Spring 5-7-2021

Deciphering the Ecology of Aulacoseira Taxa in Alpine Lakes: Implications for Paleoclimate Reconstructions

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DECIPHERING THE ECOLOGY OF *AULACOSEIRA* TAXA IN ALPINE LAKES: IMPLICATIONS FOR PALEOCLIMATE RECONSTRUCTIONS

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A DISSERTATION

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

(in Ecology and Environmental Science)

The Graduate School

University of Maine

May 2021

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An Abstract of the Dissertation Presented
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Diatoms of the genus *Aulacoseira* are thought to bloom during enhanced lake mixing. Thus, changes in their relative abundances in lake sediment records may track lake thermal conditions and be used to reconstruct past climate. However, the lack of species-specific ecological information for the genus, and taxonomical conflicts for low-height mantle taxa, generates uncertainty about the conditions in which they occur. As many of the environmental reconstructions analyzing alpine lake sediments report species from this genus, it is relevant to understand the factors that control their growth and persistence to improve climate inferences.

This research focused on low-mantle species, particularly *Aulacoseira pusilla*, to examine the ecology of this species in alpine lakes. By studying the distribution of this taxon in two alpine ecosystems in the Greater Yellowstone Area and testing the effect of light, nutrients, and

vertical distribution in the water column, we found that light controls the distribution of the population. The nutrients availability regulates the sedimentation in stratified conditions but stimulates the growth in depleted conditions.

The observation of sediment records from two alpine lakes showed that warm periods favor the relative abundance of *A.pusilla*. Thus, under present conditions, higher relative abundances were found in contemporary sediments. Relative abundances of this taxon were linked with low snow water equivalent, indicative of snowpack reduction or shifts in snowfall. These factors reduce lake insulation and increase radiation access. However, changes in light access did not induce the same response of similar low-mantle species.

The response to deeper light access were different for *A. alpigena,* suggesting that there are ecological differences in the species complex and that the impact depends on the characteristics of lake water.

Searching for *A. pusilla* in sediment records of similar lakes in the Beartooth Mountains showed that the taxon has a limited distribution in the area. However, additional studies are required.

In conclusion, this study provided information about the distribution of *A. pusilla* in the water column, identified that light access is the principal factor regulating the growth and distribution of the taxon, and showed that changes in the relative abundance indicate reductions in snow accumulation.

ACKNOWLEDGEMENTS

I want to express my gratitude to my advisor Dr. Jasmine Saros for her guidance and help to reach this goal. Her labor advising this research taught me beyond the limnological field. From her work, I learned elements that contributed to my professional growth and transcended to personal growth. Without her approval and support to start this challenge, none of the things I have experienced in this country would have been possible. I also want to thank my committee members, Dr. Hamish Greig, Dr. Ben Peierls, Dr. Kirk Maasch, and Dr. Stone, because their contribution during the research process improved this study. I cannot forget the help and collaboration of present and past students of the Saros' lab, Joseph Mohan, Vaclavá Hazuková, Matthew Farragher, Grayson Huston, Simona Lukasik, Ben Burpee, Rachel Fowler, Kate Warner, Carl Tugend III, and Heera Malik. This work was also possible by the Sawyer Water Research Lab team Tamara Levitsky, Mike Handley, and Scott Lariviere. Thanks to those CCI students and the members of the SBE that made me feel part of a team.

I reached this stage of the Doctoral studies thanks to the support of Fulbright Colombia, the Dan and Betty Churchill Exploration Fund, and the Colciencias-Fulbright Scholarship. Without their funding, the experiences of the last four years would not be possible.

I cannot forget my family. They gave me the strength to pursue this challenge and are the reason to keep walking. Finally, thanks to God, he is the one who captains my life.

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1 CHAPTER 1. INTRODUCTION

The genus *Aulacoseira* is a frequent component of diatom fossil records. It has been used to detect changes in the stability of the water column, due to the effect that mixing exerts, propelling them from bottom to surface waters to ensure light access. The morphological complexity of the low-mantle taxa generates taxonomical problems that affect the identification of the species. Relatively limited understanding of the *Aulacoseira* taxa has resulted in conflicting reconstructions and gaps in the ecology of the species. Hence, my research focused on the study of the species in the *A. distans* complex. I observed contemporary samples of *A. pusilla* to understand the factors that control the taxon, and by analyzing lake sediment cores, I aimed to determine if the use of refined taxonomy would reveal links to changes in lakes associated with snowpack. Snowpack is a factor that thermoregulates lake processes. Thus, a proxy to understand snowpack changes would represent an advance for environmental reconstructions.

In Chapter 2, to understand the ecology of *A. pusilla*, I performed lake surveys in three lakes in the Greater Yellowstone Area to assess the spatial and temporal distribution of the taxon in the water column. In addition, I conducted *in situ* incubation experiments using populations from two of the sampled lakes to understand how this species responds to light variation and nutrient availability.

In Chapter 3, changes in the relative abundances of *A. pusilla* and similar taxa such as *A. alpigena, A. perglabra, and A. nivaloides* were analyzed in relation to changes in snowpack using lake sediment cores from Kersey and Beauty lakes using refined taxonomy. I looked for

links between *Aulacoseira* taxa in the diatom assemblages and shifts in snowpack reconstructed from tree rings.

In Chapter 4, to complement the understanding of the ecology of *A. pusilla*, I examined lake sediment records in five additional alpine lakes in the area to assess occurrence patterns of *Aulacoseira pusilla*.

In Chapter 5, I summarize the main findings of this study and mention the perspectives that future research that can address from the contributions of the research.

2 CHAPTER 2. ECOLOGY OF *AULACOSEIRA PUSILLA* IN OLIGOTROPHIC MOUNTAIN LAKES: CHALLENGES TO THE CLASSIC PARADIGM

2.1 Abstract

We investigated the distribution and ecology of the planktonic diatom *Aulacoseira pusilla* (F.Meister) A.Tuji & A.Houk (2004) in three high elevation lakes of the US Central Rocky Mountains. Vertical distributions of *A. pusilla* populations in each lake were quantified every 3–4 days for three weeks during July 2017, shortly after the ice-out period. Populations from two of the lakes were incubated *in situ* in two different experiments, to assess the effects of incubation depth × nutrient additions and to quantify responses to light. Spring turnover is considered the blooming period of planktonic *Aulacoseira* taxa typical of oligotrophic lakes. We found that in stratified conditions, the vertical distributions of this species varied spatially and temporally across the three lakes over the month of July. There were blooms in the epilimnion of a moderately transparent lake, and in deeper waters of a clear lake. The patterns of *A. pusilla* in the lakes and experiments suggest that light is one of the factors controlling the ecology of this taxon, whereas nutrients did not show a clear trend. We provide evidence that challenges the paradigm of *Aulacoseira* as an indicator of lake turnover, and suggest the need for more taxon-specific quantification of ecology.

2.2 Introduction

The genus *Aulacoseira* Thwaites, 1848, is considered cosmopolitan in distribution, with recorded observations on every continent except Antarctica (Kociolek, 2018). These centric diatoms have cylindrical valves with an expanded mantle and heavily silicified frustules attributed to ornamented cell walls (Edgar, 2003). Cells can occur individually, or in straight colonies connected by interlocking spines at the margin of the valve face (Wehr et al., 2015). *Aulacoseira* has been reported in a wide variety of conditions from nutrient-limited to eutrophic lakes, in low alkaline to dystrophic waters, and in turbid to clear lakes. For taxa commonly associated with lower productivity lakes (e.g*., A. pusilla, A. distans, A. subarctica, A. alpigena. A italica*), the ecological paradigm centers on lake thermal structure, and suggests that these diatoms typically bloom during periods of enhanced lake mixing (Carrick et al., 1993; D. Jewson, 1992; Lund, 1954, 1971; C. Reynolds & Irish, 1997). (C. S. Reynolds et al., 2002) place *A. subarctica* in their Codon B functional group, representative of vertically mixed conditions with low light and moderate nutrients. The inference is that mixing events suspend these heavily silicified cells and also increase advection of relatively nutrient-rich hypolimnetic water, whereas this species can form long, relatively dense cell chains that sink rapidly during periods when lakes are stratified, encysting on the lake sediment surface between mixing events (Sicko-Goad et al. 1986, McQuoid and Hobson 1996).

While *Aulacoseira* taxa are a widespread and common member of lake phytoplankton, our understanding of the ecology of this group is based on a rather limited number of studies focused on quantifying the response of these species to environmental factors such as light, nutrients, or their interaction (Carrick et al., 1993; Foy & Gibson, 1993; D. Jewson et al., 1981).

The effect of light was addressed by Foy and Gibson(Foy & Gibson, 1993), who found a low growth efficiency of *A. subarctica* at sub-saturating irradiances; growth kinetics at low light for this taxon are temperature-independent (C. Gibson & Foy, 1989). Nutrient responses do not present a clear pattern. Contemporary studies of *A. alpigena* in glacial lakes in North America showed a positive response to N addition and no effect of P (Williams et al., 2016a). In humic reservoirs, Tuji (Akihiro Tuji, 2015) observed *A. pusilla* in mesotrophic lakes, but Gibson (C. E. Gibson et al., 2003) reported diminished populations in lakes with total phosphorus higher than 3.3 µM.

Understanding the ecology of *Aulacoseira* taxa is further complicated by taxonomic issues and identification difficulties. English and Potapova (Jonathan English & Potapova, 2009) highlighted this issue, pointing out that misidentification among several low-mantle *Aulacoseira* taxa leads to unreliable datasets and is confounding our ecological understanding of this group. Denys (Denys et al., 2003) specifically pointed out the uncertainty about the ecology of *A. subborealis* given the misidentification with *A. subarctica*. Similarities in valvar traits can lead to misidentifications among *A. subborealis, A. subarctica, A. alpigena, A. laevissima, A. nygaardii, A. distans, A. nivalis, A. nivaloides* and *A.pusilla* or simply lead to grouping them together under the *A. distans* complex. Such uncertainty impacts studies about the distribution of species and casts doubts on the environmental reconstructions derived from ecological interpretations. For example, *A*. *alpigena* is associated with acidic oligotrophic environments, whereas *A. pusilla*, which is sometimes confused with *A*. *alpigena,* has been reported from a wide variety of trophic conditions (Denys et al., 2003; Leira, 2005; Akihiro Tuji, 2015). Indeed, in re-examining *Aulacoseira* taxa from sediment cores in the US Central Rocky Mountains (Stone et al. 2016), we

determined that valves originally denoted as *A. alpigena* are more accurately identified as *A. pusilla* (S. A. Spaulding et al., 2020).

The relationship between *Aulacoseira* and lake mixing is used extensively in paleolimnological reconstructions. For example, extended lake mixing periods and delayed ice out have been inferred from increases in the relative abundances of various *Aulacoseira* taxa, with the effects of these factors on *Aulacoseira* ecology confirmed in some studies using contemporary water column patterns (Ruhland et al., 2008; Solovieva et al., 2015; Wang et al., 2008). Stone (Stone et al., 2016a), using shifts in *Aulacoseira* densities as well as other diatom taxa, constructed a Diatom-Inferred Stratification index (DI-SI) to identify periods of deep and shallow mixing in alpine and glacier lakes. The reliance on *Aulacoseira* taxa for these inferences suggests a need to better understand the ecology of this widespread group.

The information about the ecology of *Aulacoseira* is limited, based primarily on distribution patterns, and complicated by taxonomic issues and misidentifications. The objective of this research was to identify the environmental factors that control the occurrence and distribution of *Aulacoseira* in oligotrophic lakes. Specifically, we asked whether distributions and densities of an *Aulacoseira* species, *A. pusilla,* are affected by changes in water column stability or are a result of the interaction of physical aspects of the water column and nutrient availability. To address this question, the vertical and temporal distributions of *A. pusilla* were studied in the water columns of three high-elevation lakes of the Greater Yellowstone Area during July 2017. *In situ* microcosm experiments were used to assess the effects of nutrient availability, light access, and depth in the water column on the growth of this

species. The findings of this study are significant because they provide information about the ecology of *A. pusilla,* a common but neglected planktonic taxon (Denys et al. 2003).

2.3 Methods

2.3.1 Site description

The Absaroka-Beartooth area is located in the central Rocky Mountains of the US in Montana and Wyoming (**Figure 2.1**). The geology of this area is dominated by Precambrian granitic rock composed of Archean Gneiss and accessory metamorphic rocks (Love & Christiansen, 1985). The landscape is characteristic of the Greater Yellowstone Area, with alpine plateaus and U-shaped valleys; tree lines in the region range between 2750 to 3000 m above sea level (Saros et al., 2003). Because of the continental climate, the area has the lowest but longest snow accumulation (peaking in April) for the Western U.S, and the latest snowpack ablation season, which occurs around June (Trujillo & Molotch, 2014). The region holds around 600 permanent alpine or sub-alpine lakes, characterized by low concentrations of phosphorus. The ice-free season in these lakes spans from early July to October.

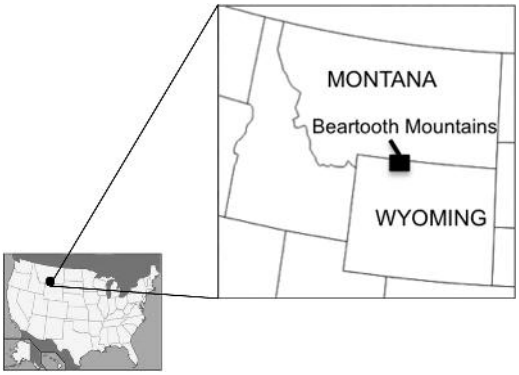


Figure 2.1 Location of the study lakes

Three lakes were selected for this study (Beauty Lake, Beartooth Lake, and Kersey Lake) based on previous research that indicated the presence of various *Aulacoseira* taxa in these lakes (Saros et al., 2003; Stone et al., 2016a). Beauty (44.97°N, 109.57°W) and Beartooth (44.95°N, 109.60°W) lakes are situated at treeline, while Kersey (45.03°N, 109.84°W) lies in a forested watershed. The lakes are deep (>20 m) with small surface areas (<1 km^2). The waters are oligotrophic, circumneutral (pH 6-7.6), clear (1% attenuation depth for photosynthetically active radiation ($Z_{1\%PAR}$) 10-17 m), and have low conductivity (7 to 30 µS cm^{-1}) (**Table 2.1**). While we were targeting a variety of *Aulacoseira* taxa with our sampling, we primarily and consistently found *A. pusilla* in the three study lakes, hence focus on this taxon for this work.

Table 2.1 Select parameters of the study lakes.

Maximum depth (Z_{Max}), depth at which 1% of surface photosynthetically active radiation remains ($Z_{1\%PAR}$), Total Phosphorus (TP), Soluble Reactive Phosphorus (SRP), Soluble reactive silica (SRSi). Data from Saros et al. (2003, 2012), Kessler et al. (2008).

	Beauty	**Beartooth**	**Kersey**
Coordinates	44.97°N, 109.57°W	44.95°N, 109.6° W	45.95°N, 109.54°W
Altitude (m.a.s.l)	2874	2713	2460
Lake Area (Km^2)	0.36	0.45	0.47
Z_{Max} (m)	35	26	21
Mixing depth (m)	4	5	5
$Z_{1\%\ PAR}$ (m)	>17	12.8	10
Nitrate (µM)	< 0.07	<0.07	< 0.07
TP (µM)	0.1	0.1	0.06
SRP (µM)	< 0.02	<0.02	<0.02
DSi (µM)	28.7	27.2	-

2.3.2 Distribution patterns

The ecology of *Aulacoseira pusilla* was assessed by repeat sampling of vertical distributions of the diatom across the three study lakes shortly after ice-out and by conducting *in situ* incubations in which light and nutrients were manipulated.

The vertical distributions of *A. pusilla* were assessed in each lake by surveying every three days between July 11 to 28 2017. Water was collected with a Van Dorn bottle every 3 m down to a depth of 15 m in Kersey Lake, 18 m in Beartooth Lake, and 21 m in Beauty. The maximum depth of sampling was informed by relative differences in water clarity (e.g., Kersey Lake least

transparent). Two 50mL sub-samples were collected from each depth and preserved with Lugol´s iodine for phytoplankton enumeration. Vertical profiles of temperature (°C), conductivity (SPC), dissolved oxygen (DO), and pH were measured with a YSI EXO2 multi-parameter sonde at 1-m intervals down to a depth in the hypolimnetic zone of each lake where conditions remained constant. The identification of the epi-, meta-, and hypolimnion was determined by the temperature profile, based on the metalimnion as the zone in which temperature changed by ≥1°C per meter.

Water from each zone was analyzed for total phosphorus (TP), total nitrogen (TN), soluble reactive phosphorus (SRP), dissolved organic carbon (DOC), nitrate (NO_3^-), ammonium (NH_4^+), and dissolved silica (DSi). Samples for the various dissolved nutrients and DOC were filtered with 25-mm Whatman GF/F filters (0.7-µm pore filter) pre-rinsed with deionized water, except DSi, which was filtered with polycarbonate filters (0.4-µm pore filter). Total nutrients were analyzed on whole water samples. TP and TN were determined using the persulfate digestion method (APHA, 2000). NO_3^- was measured with the cadmium reduction method and NH_4^+ with the phenate method. The nitrogen fractions were run on a Lachat QuickChem 8500 flow-injection analyzer with a limit of quantification of 0.07 µM N for NO_3^-, 0.14 µM N for NH_4^+, and 0.7 µM N for TN. Values for NO_3^- and NH_4^+ were added to determine dissolved inorganic nitrogen (DIN). SRP was analyzed with the ascorbic acid method. Both TP and SRP were analyzed on a Varian Cary-50 spectrophotometer, both with a limit of quantification of 0.06 µM P. DOC was measured using a Shimadzu TOC analyzer.

Light penetration in the water column was measured using a Secchi disc, approximating the $Z_{1\%PAR}$ depth, assumed to be double the depth of the Secchi reading. Values of $Z_{1\%PAR}$ were used to calculate the vertical extinction coefficient of light intensity (K_d) for each sampling date. The average PAR in the epi-, meta, or hypolimnion was calculated using the equation (Tilzer & Goldman, 1978):

$$\acute{I}_{Z_a \to Z_b} = \frac{I_0}{K_d \; Z_b - Z_a} \; e^{-k_d Z_a} - \; e^{-k_d Z_b}$$

where Z_a= depth at the top of the layer, Z_b= depth at the bottom of the layer, I_o= irradiance at the surface, assumed to be equal to 1800 µM m^{-2} s^{-1}, K_d= attenuation coefficient.

The thermal stability of the water column on each date in each lake was determined by calculating the Relative Thermal Resistance (RTR) that measures the energy required to mix water of different densities (due to different temperatures) (Birge, 1910):

$$RTR = \frac{p2 - \; p1 \; 10^6}{8}$$

where *p2*= water density at the bottom using the average temperature in the hypolimnion for each sampling date, *p1*= water density at the surface using the average temperature in the epilimnion for each sampling date.

Phytoplankton samples from the vertical profiles were counted by the Utermöhl method, using 30 mL of each sample. The phytoplankton community was identified and enumerated with a Nikon TS-100 inverted microscope with 400X magnification. The counting of one transect established the community composition, and four additional transects were counted exclusively for the abundance of *A. pusilla* to improve the accuracy of these counts. One replicate per depth per sample date was counted.

2.3.3 Experimental approaches

Two experiments were conducted to assess the response of *A. pusilla* to nutrients and/or light, one in which nutrients and incubation depth were manipulated and the other in which light exposure was varied at a fixed depth in the water column. The latter was conducted to isolate the effects of light from other factors (e.g., temperature) that could vary with the depth of incubation. Each experiment was conducting using two sets, one set with *A. pusilla* populations from Beauty Lake, and another set with those from Kersey Lake. All experiments were incubated in Beauty Lake to ensure the same light and temperature conditions and because Beauty Lake has greater water clarity than Kersey Lake. All incubations were carried out in 75-mL non-treated culture flasks in triplicate. For each lake, 5 L of water were collected from the middle of the epi-, meta, and hypolimnion zones using a Van Dorn bottle to produce an integrated sample that was filtered with a 153-µm Nitex mesh to remove zooplankton.

The effects of nutrients and incubation depth on *A. pusilla* growth and cell densities were assessed in a 2 × 3 experimental design that tested nutrient addition (N+P or no addition) and location in the water column (epi-, meta-, or hypolimnion). Nutrient additions were in the form of $NaNO_3$ (8 µM N) and NaH_2PO_4 (1 µM P). Flasks were incubated along an anchored rope attached to a buoy, with flasks placed into transparent Bitran bags, which were then clipped onto the cord at 2 (epilimnion), 8 (metalimnion) or 15 m (hypolimnion) depth to position them in the appropriate zone. The incubation lasted eight days from July 14 to 22. Solar irradiance was not measured; however, HOBO sensors were used to track the temperature and relative light at incubation depths. The sensors were attached to the ropes close to the experimental units at each depth.

The effect of light on *A. pusilla* growth and cell densities was assessed at 100%, 65%, and 25% of ambient PAR exposure. Flasks were all incubated in the epilimnion at 2 m with light exposure unmodified (100% of ambient), or modified by covering the flasks with window screen (65% of ambient) or window screen + mesh bag (25% of ambient), according to the procedure described by Malik (Malik et al., 2017). All flasks were individually placed inside a transparent Bitran bag and clipped to an anchored rope. The containers were incubated for eight days (from July 14 to 21).

Phytoplankton samples were counted as described above, with two flasks per treatment counted for the incubations.

2.3.4 Data analyses

Cell densities (cells mL^{-1}) were calculated using count data. The total density for *A. pusilla* in the epi-, meta- or hypolimnion on each date in each lake was calculated by the sum of the density values for each of the samples associated with the zone. The average pH, temperature, SPC, and DO were also calculated for each zone in each lake. Visual assessment of quantile-quantile graphs (package ggplot) and the outcome of Shapiro-Wilk tests (library nortest (Gross & Ligges, 2015) were used to verify normal distribution of data. Since not all variables met the assumption of normality, a pair plot with Spearman´s rank-order correlation was run, including all the variables (package psych (Revelle, 2019). The analysis was repeated for each lake dataset using Rstudio version 3.6.2 (R Core Team, 2019).

To understand the effect of the variables on the density of *A. pusilla*, we ran a regression analysis using Generalized Linear Interactive Modeling (GLM). The continuous variables included for the GLM were the biologically relevant factors identified from the Spearman´s rank-order correlation. Even though we found variables that correlated to each other (light, temperature, pH and DOC), variable selection was based on the physiological effect on phytoplankton. The GLM was run using the Log link and the Poisson distribution. The test was run separately for each lake given that relevant variables were different for each lake. Kersey and Beauty data included average values for conductivity (SPC), light (PAR), and nutrient concentration (TN, TP) in each zone (epi, meta or hypolimnion) to test the association with *A. pusilla* density (den). For Beartooth Lake, the analysis was executed with SPC, TP, PAR and ammonium (NH_4). The test was run without testing interaction between the terms. When overdispersion or underdispersion was detected using the package AER (Kleiber & Zeileis, 2008), the distribution was adjusted with Quasi-Poisson dispersion correction. The fitted models were proposed checking the residuals using the package car for R (Fox & Weisberg, 2019) and by the removal of insignificant variables and checking the effect on deviance. The results of these analyses were graphed with the package jtools (Long, 2019). We plotted the standardized regression coefficients.

For each experiment, count data were transformed to density (cells mL^{-1}), and growth rates were calculated using the standard equation:

$$\mu = \frac{ln\ F\ -\ ln\ I}{T}$$

where F = final density, I = initial density, and T= number of incubation days. The values of density and growth rate for each set of experiments were graphically analyzed using box plots

(package ggplot2 (Wickham, 2016)) to detect trends for nutrient addition and incubation depth. Using the Shapiro-Wilk test and quantile-quantile plots, the normality assumption for density and growth rate was examined. Levene`s test (Package car (Fox & Weisberg, 2019)) was used for checking the homogeneity of variance.

A two-way ANOVA was used to assess the effects of nutrients and incubation depth on growth rate, as well as cell densities. One-way ANOVA was used to evaluate the effect of light on density and growth rate in the light manipulation experiment. Both analyses were performed using the library rstatix for R. Normality assumptions for each ANOVA were analyzed using model residuals. When the ANOVA showed significant effects, post-hoc comparisons were executed (Tukey`s Honestly Significant Difference- HSD) to identify the treatments and the differences between the levels in the treatments. The post hoc comparisons were ran using the package agricolae (De Mendiburu, 2020). All the analyses were executed in R studio, R version 3.6.2 (R Core Team, 2019).

2.4 Results

2.4.1 Distribution patterns

The thermal structures of the three lakes followed lake distributions along the elevational gradient. Kersey Lake, situated at the lowest elevation, was already thermally stratified when sampling began on July 10 **(Figure 2.2),** with surface waters at 18˚C and epilimnion depth (defined as the top of the epilimnion) at 2 m. Epilimnion depth ranged from 2 to 3 m over the sampling period; the limit of the metalimnion ranged from 7 to 9 m over the period. RTR values were higher (i.e., greater thermal stability) than those of the other two lakes

and remained high throughout the sampling period. In Beauty Lake, situated at the highest elevation of the three lakes, initial surface temperatures on July 11 were around 9˚C, and the lake was not thermally stratified. By July 14, the lake was weakly stratified at 5 m; epilimnion depths ranged from 2 to 3 m over the rest of the sampling period, and the limit of the metalimnion ranged from 5 to 7 m. RTR gradually increased over the sampling period (Supplementary **Figure 7.1**). Beartooth Lake, situated at an elevation between the other two lakes, was also already thermally stratified when sampling began on July 11, with surface waters at 13˚C and epilimnion depth at 3 m. Epilimnion depth ranged from 2 to 3 m over the sampling period; the limit of the metalimnion ranged from 5 to 8 m over the period. RTR gradually increased over the sampling period (Supplementary **Figure 7.1**).

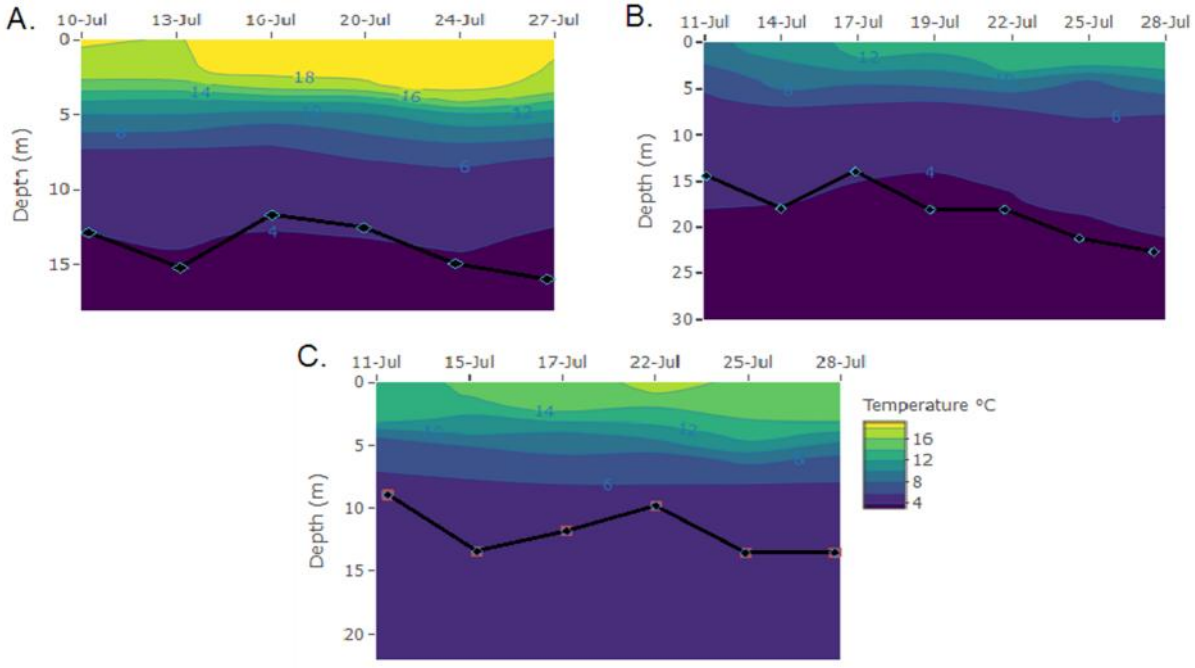


Figure 2.2 Temperature profile (colored contours) and Z1%PAR depth.

Temperature profile (colored contours) and Z1%PAR depth (solid black line) between July 11 to 28, 2017, in A) Kersey Lake, B) Beauty Lake, C) Beartooth Lake.

Water clarity was generally high but varied across all lakes (**Figure 2.2**). Calculated $Z_{1\%PAR}$ for Kersey Lake suggests that the limit of the photic zone was between 12 to 14 m (**Figure 2.2 a**). Water clarity was highest in Beauty Lake, with $Z_{1\%PAR}$ at 15 m or greater **(Figure 2.2 b).** In Beartooth Lake, the $Z_{1\%PAR}$ was the lowest of the lakes, fluctuating around 10 m.

Nutrients were generally low, but fluctuated spatially and across all lakes (**Table 2.2**, Supplementary **Figure 7.2** a–c). TP was <0.2 µM in all lakes, indicating oligotrophic systems. The lowest values were in Beauty Lake (0.1 µM). DIN was low but variable across lakes and zones. The lowest DIN concentrations were often in the epi- and metalimnion of all lakes, and the highest values were often found in the hypolimnion, but there were some exceptions, yielding highly variable DIN: TP over the sampling period (Supplementary **Figure 7.2** d–f). In Kersey Lake, N and P co-limitation was often found in the epi- and metalimnion, except on July 13 when N limitation was indicated. There were occasional indications of P limitation in these zones as well over the study period. The hypolimnion was the only layer that displayed a consistent pattern of P limitation during the study. DIN:TP and inferred limitation patterns were variable in Beauty Lake. In Beartooth Lake, limitation patterns also appeared variable, but the hypolimnion always appeared P-limited.

The phytoplankton communities varied across the three lakes. The Kersey Lake community was dominated by *Dinobryon* sp (57%), Beauty Lake by *Asterionella formosa* (55%), and Beartooth Lake was co-dominated by *Rhodomonas* sp (42%). and *Asterionella formosa* (40%). The genus *Aulacoseira* represented less than 5% of phytoplankton density in each lake over the total sampling period (Supplementary **Figure 7.3**).

Table 2.2 Average values of physical and chemical variables in the different lake strata for the study lakes from July 11 to 28, 2017

		Temp (°C)	SPC (µS cm^{-1})	pH	DIN (µM N)	TN (µM)	TP (µM)	DOC (mM)	Avg PAR (µM m^{-2} s^{-1})	$Z_{1\%\ PAR}$ (m)
Kersey	**Epi**	18.2	19	8.0	0.6	7.9	0.1	217	1136	
	Meta	8.9	20	7.7	0.6	6.6	0.2	208	295	14
	Hypo	4.1	21	7.3	1.6	7.9	0.2	192	35	
Beauty	**Epi**	11.3	13	8.1	0.6	3.6	0.1	67	1254	
	Meta	6.6	13	8.0	0.7	3.9	0.1	83	386	18
	Hypo	3.9	13	7.5	1.6	4.4	0.1	58	24	
Beartooth	**Epi**	13.6	33	8.1	0.7	5.0	0.2	100	1040	
	Meta	6.3	34	7.9	0.6	4.9	0.2	125	131	11.4
	Hypo	4.6	39	7.6	2.2	7.1	0.2	108	7	

The densities and distributions of *A. pusilla* showed divergent patterns across the lakes. In Kersey Lake, cell densities oscillated between 0.3 to 29 cells ml^{-1} and were low throughout the water column until mid-July (July 16). After that, the species increased and peaked in the epilimnion (**Figure 2.3a**). Beauty Lake had the highest densities of any lakes (1.5–42 cells ml^{-1}; **Figure. 3b**). At the beginning of the study (July 14), higher densities were found in the hypolimnion from about 8 to 21 m, with a peak in density at 18 m. Cell densities were low on July 17, and then a bloom occurred on July 19 with the highest densities in the upper hypolimnion

between 9 to 15 m; this peak persisted until the end of the study. The lowest densities of this diatom were found in Beartooth Lake (maximum of 12 cells ml^{-1}), where the highest density occurred in the metalimnion during the second half of July (**Figure 2.3c**).

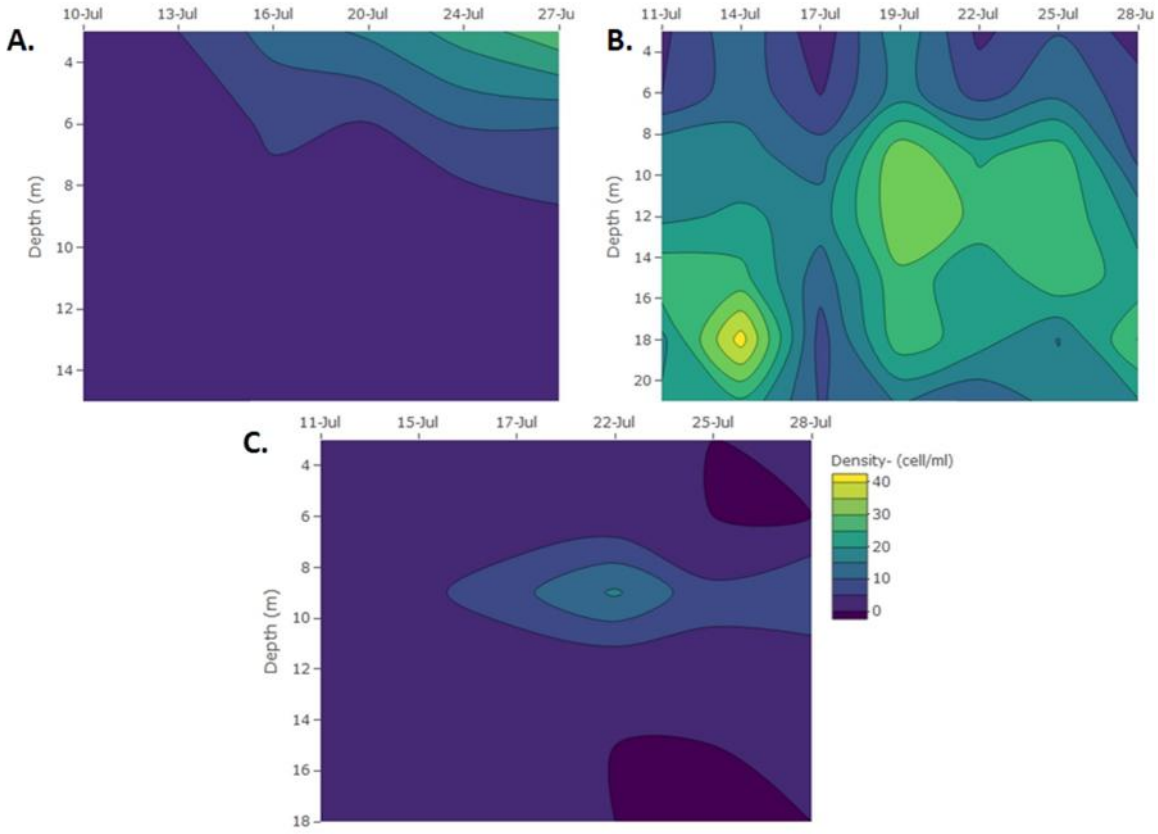


Figure 2.3 Vertical profile of distribution of *Aulacoseira pusilla* between July 11 to 28, 2017. A) Kersey Lake, B) Beauty Lake and C) Beartooth Lake. Note difference in depth scales across the lakes.

The regressions revealed that light was the common factor affecting the distributions of *A. pusilla* in all lakes, but the magnitude of the effect varied (**Figure 2.4**). In Kersey Lake, higher light had a positive impact on the density of *A. pusilla* ($1.16e^{-3} \pm 3.9e^{-4}$, $p<0.05$), whereas the opposite was found for Beauty ($-1.8e^{-3} \pm 1.3e^{-3}$, $p<0.001$) and Beartooth lakes ($-4.9e^{-3} \pm 1.8\ e^{-3}$,

p<0.05). In Beartooth Lake, conductivity was also important (-0.45 ± 0.2, p<0.001) with cell densities higher when conductivity was lower. In Kersey and Beartooth lakes, models explained ~40% of the variability and 65% for Beauty Lake (Supplementary Table SI).

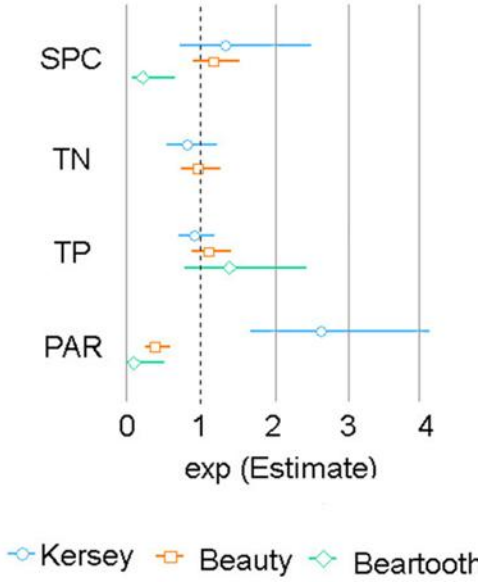


Figure 2.4 Forest plot of model coefficients for Kersey, Beauty and Beartooth cell densities for *Aulacoseira pusilla* regressions

2.4.2 Experimental approaches

The average densities of *A. pusilla* at the beginning of the experiments for Kersey and Beauty lakes were 3.4 and 3.8 cells ml^{-1}, respectively. Lake water chemistry at the time of collection of the experimental water and populations is provided in Table III. The average temperatures during the course of the experiments were recorded by HOBO sensors. In the epilimnion it was 12.5°C ± 0.3, 6°C ±0.1 in the metalimnion, and 4°C ± 0.02 in the hypolimnion. Average irradiance (I_z) at each incubation depth was 1392 µM m^{-2} s^{-1} at 2m, 525 µM m^{-2} s^{-1} at 8m, and 97µM m^{-2} s^{-1} at 15m.

In the nutrient × incubation depth experiments, responses of *A. pusilla* populations from Kersey Lake (**Figure 2.5** a & b) and Beauty Lake (**Figure 2.5** c & d) were similar. Both populations had the highest cell densities and growth rates in the epilimnion incubations. The interaction of the two factors did not influence the density (Kersey: $F(2,12=1.9)$, $p=0.18$, effect size 0.24; Beauty: $F(2,12= 0.35)$, $p=0.7$, effect size =0.05), nor the growth rate (Kersey: $F(2,48 =1.26)$, $p=0.3$, effect size 0.05; Beauty: $F(2, 48 =1.25)$, $p= 0.2$, effect size 0.05) in either lake. In Kersey Lake, nutrient addition contributed 40% of the density values ($F(2,12=8.1)$, $p<0.05$, effect size 0.40) and 27% of the growth rate ($F(1,48=18.16)$, $p<0.05$, effect size 0.27). Incubation depth had an effect of 75% on density ($F(2,12=18.2)$, $p<0.05$, effect size 0.75), and 59% for growth rate ($F(2,48=34.3)$, $p<0.05$, effect size 0.59). Post-hoc comparisons revealed that both nutrient treatments and incubations depths were statistically different for density and growth rate. In Beauty Lake, incubation depth was the only factor that had an effect on *A. pusilla* density and it was responsible for 53% of the response in density ($F(2, 12 = 6.85)$, $p<0.05$, effect size = 0.53) and 43% of the growth rate ($F(2, 48=18.5)$, $p<0.05$, effect size= 0.43). Nutrient addition was not significant for cell density ($F (1,12=0.012$, $p >0.05$) nor growth rate ($F(1, 48= 5\ e^{-4)})$, $p= 0.9$). Post hoc comparisons indicated that the greatest difference in densities occurred between the hypo- (4.1, ±0.81) and epilimnion (10.1, ±3.3). A similar pattern was observed for the growth rate between the epi- (0.11, ±0.05) and hypolimnion ($7.7\ e^{-3}$, ±0.04), while the growth rate in the metalimnion (0.07 ±0.06) was not different from the epilimnion.

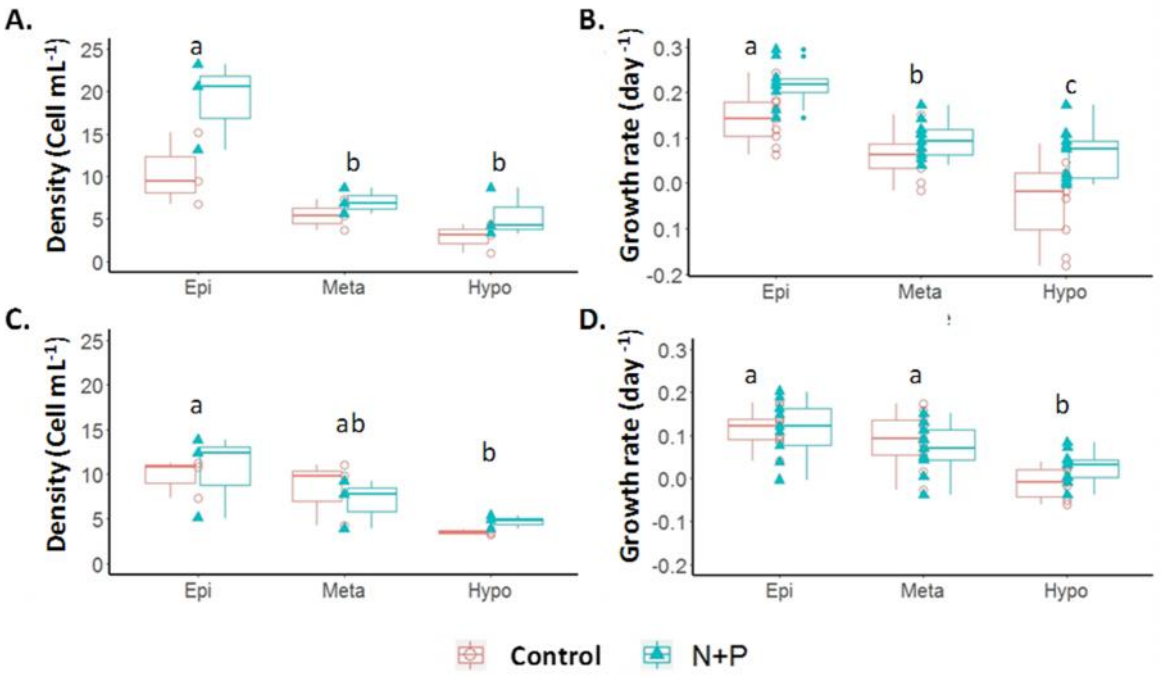


Figure 2.5 Response of *Aulacoseira pusilla* to incubation depth and nutrients in bioassay experiments.

A) cell density and B) growth rate in Kersey Lake; C) cell density and D) growth rate in Beauty Lake. Box ends are 25th and 75thpercentiles; bold bars within boxes are 50th percentiles. Letters inside plots denote the results of post-hoc analyses. Open circles are Ctrl treatments, closed triangles are N+P treatments; closed circles represent outliers.

In the light experiments with Kersey Lake populations, there were no significant differences in cell densities across light levels ($F(2, 6 = 1.31)$, $p=0.34$, effect size=0.34; Fig. 6a), but light had a positive effect on growth rates (Fig. 6b). Higher growth rates were apparent in the 100% and 65% ambient PAR treatments compared to those exposed to 25% ambient PAR. The ANOVA indicated significant differences in the growth rate of the light treatments ($f (2, 24 = 3.4)$, $p p<0.05$, effect size 0.24). However, post-hoc comparisons did not identify statistical

differences between the treatments (100% PAR: 0.14 ± 0.04, 60% PAR: 0.13 ± 0.07, 25%PAR: 0.07± 0.05). There were logistical problems with the experiments with Beauty Lake populations (relative light intensity data on the HOBOs indicated that some flasks floated higher in the water column after anchoring), so these samples were discarded from the study.

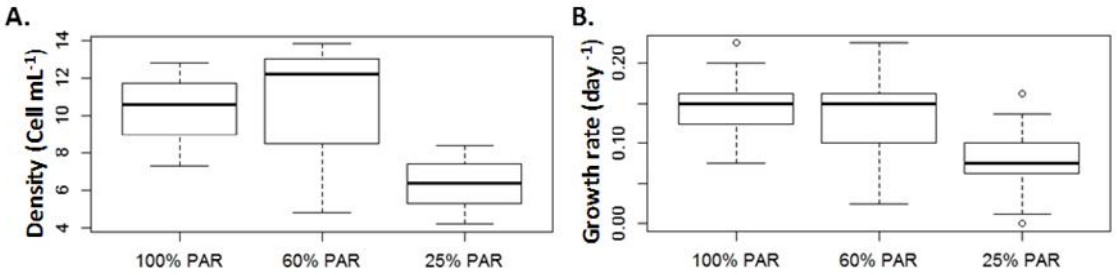


Figure 2.6 The response of *Aulacoseira pusilla* from Kersey Lake to different levels of light expressed as a percent of ambient (100%, 60%, or 25%) at 2-m depth.

2.4.3 Discussion

With much prior research suggesting an association between *Aulacoseira* and lake mixing (Carrick et al., 1993; Gibson et al., 2003; Ilmavirta & Kotimaa, 1974a; Jewson et al., 1981; Pla et al., 2005), and our study starting with stratified lake conditions, we expected to find *A. pusilla* limited to deep zones of the three high-elevation lakes. However, the spatial and temporal variation that we found across these three lakes during July 2017 suggests that for these populations of *A. pusilla*, turbulence-induced advection of cells is not the primary driver of vertical and temporal distributions. These distribution patterns, together with the experimental results, suggest the importance of light in shaping the response of this taxon. They also suggest that light requirements may vary across populations of *A. pusilla* based on ambient light and nutrient conditions. Specifically, in the same type of nutrient × incubation

depth experiments, the Beauty Lake population was unaffected by nutrient enrichment, suggesting that it had sufficient nitrogen and phosphorus to support growth. This population grew equally well in the light conditions of the epi- and metalimnion. In contrast, growth of the Kersey Lake population was stimulated by nutrient addition, suggesting nutrient-depleted conditions in Kersey Lake. Growth rates of this population were greatest in the higher light conditions of the epilimnion, suggesting that nutrient depletion may have constrained growth at lower light intensities. The differing light requirements of these two populations were also reflected in vertical distribution patterns of these two lakes over July. Collectively, these results suggest that for *A. pusilla*, as with many other diatom taxa (Malik & Saros, 2016; Saros et al., 2016), relationships with lake physical features (e.g., mixing) are in part owing to links with effects on resource (light, nutrient) availability.

The distributions of other *Aulacoseira* taxa also highlight the importance of light in explaining distribution patterns (Foy & Gibson, 1993; C. E. Gibson et al., 2003; C. Gibson & Fitzsimons, 1990; D. Jewson et al., 1981). Jewson (D. H. Jewson et al., 2009) found that light attenuation regulates vertical distribution and growth rates of *Aulacoseira baicalensis,* diminishing its occurrence at shallow depths and at light intensities that induce inhibition, but allowing its growth at deeper zones or optimal illumination. According to this, the vertical distribution patterns are not only a product of sedimentation but also the effect of changes in light access. Light attenuation affects the distribution in the water column by reduction of the energy available, while sinking decreases exposure time as well. Initiation of vertical displacement has been identified as the signal for ending the exponential growth phase (Smetacek, 1985a). Studies on *A. subarctica* found that maximum growth occurred after 12 to

20 h of continual light exposure (in light saturated conditions of 155 µM m^{-2} s^{-1}); Incubations studying the effect of mixing revealed that *Aulacoseira* division rate was significantly lower once mixing stopped than under turbulent conditions (Foy & Gibson, 1993; McCausland et al., 2001). Therefore, it is clear that the dynamics of *Aulacoseira* taxa are regulated by light exposure and the complex turbulence driven factors (nutrient availability and location in water column), which we are only beginning to understand in freshwater systems.

The mechanisms that *Aulacoseira* taxa employ to control buoyancy are another facet of these dynamics, and apparently were used in Beauty Lake, where an organic layer was observed on *A. pusilla* valves. Nutrient depleted conditions in surface waters induce sinking by diatoms, which can promote the formation of a mucous secretion (Smetacek 1985). This organic layer facilitates sinking by aggregate formation; it can change to reduce sinking rates when there are enough nutrients available or when *Aulacoseira* are in a stationary growth phase (Smetacek, 1985a; Vieira et al., 2008). The presence of an organic layer on *A. pusilla* cells in Beauty Lake, which was observed for many cells at greater depths, suggests that these populations were employing this strategy to slow down the drag effect of sedimentation, and ensure the continuity of nutrient delivery to the cells by the finding of areas with high nutrient concentration (Karp-Boss et al., 1996; Smetacek, 1985b; Titman & Kilham, 1976). The experimental results support this theory, as Beauty Lake populations did not respond to nutrient additions, indicating greater nutrient sufficiency than in Kersey Lake populations. This suggests that the deeper distributions of *A. pusilla* in Beauty Lake were likely controlled by a combination of greater nutrient availability coupled with sufficient light. In contrast, the Kersey lake pattern (late July bloom) may have been restricted to the higher light conditions of surface

waters given the nutrient depleted conditions. Studies about the effect of irradiance on phytoplankton sinking rate in *Chaetoceros gracilis* and *C. flexuosum* pointed out that sinking rates are lower in low light environments than in higher light conditions, because the growth and photosynthesis in these environments occurs at a lower rate (Culver & Smith Jr, 1989).

With our sampling beginning shortly after ice out but with thermal stratification at different stages, we may have missed earlier *A. pusilla* blooms in some of the lakes. In particular, Kersey Lake was already strongly stratified at the start of our sampling; as the lowest elevation lake in this study, ice-off was likely at least one to two weeks prior to our first sampling date. Regardless of earlier blooms in this lake, the late July bloom in the epilimnion suggested a population requiring relatively high light under the low nutrient conditions was present, which was further supported by the results of the light experiments (no nutrients were added in that set of experiments). The observation that Beauty Lake populations also shifted to shallower depths as summer progressed also supports this light and nutrient interplay, as these alpine lakes often receive a pulse of nitrogen with snowmelt, with the availability of that nitrogen rapidly declining within days to weeks after ice out (Saros et al., 2005).

Prior research has indicated that *A. pusilla* may respond to nutrient enrichment (Denys et al., 2003; Williams et al., 2016b). The July 14 peak in density in Beauty Lake coincided with high NO_3 concentration in the hypolimnion; this is consistent with observations from other alpine lakes of the US where nitrogen enrichment stimulated the growth of this taxon (Williams et al., 2016b). However, our GLM result did not broadly corroborate the link across our samples. Indeed, we cannot suggest sensitivity for N or P because, in our lakes, even though nutrient concentrations were highly variable temporally and spatially, we found no association

between nutrients and *A. pusilla* density. Furthermore, the nutrients × incubation depth experiments did not indicate a consistent, positive effect on growth in both lakes. Different nutrient status for Kersey and Beauty samples likely shaped the results for both sets. These results largely revealed the interaction with physical factors that operate along the water column, affecting the growth of this species. Studying growth rate alone should not be used to provide information about algal physiological requirements because the growth rate reflects the interaction between nutrient availability, light, and temperature (Coles & Jones, 2000; Rhee & Gotham, 1981; Staehr & Sand-Jensen, 2006).

Temperature is a factor that co-varied with light in both the distribution pattern studies and experiments, and therefore may be a partial driver of observed patterns. In Kersey Lake, *A. pusilla* developed in warmer, high light and nutrient-depleted conditions; meanwhile, in Beauty Lake, it thrived in cold, lower light and high nutrient waters. These contrasts in the initial conditions of the taxon at the onset of incubations showed that nutrient access is an important aspect that controls growth. However, there is a range of temperatures in which growth is not nutrient-limited, but temperature controlled. The growth lag at deeper depths of Kersey incubations and in Beauty samples suggest that sub-optimal conditions for this species occurred below 6°C. Warm water temperature stimulates photosynthetic activity, the nutrient demand, and boosts the growth; meanwhile, sub-optimal thermal conditions slow nutrient uptake, affecting algal growth as well (Davison, 1991; Rhee & Gotham, 1981; Staehr & Sand-Jensen, 2006). Rhee and Gotham (Rhee & Gotham, 1981) found that under sub-optimal temperature, *Asterionella formosa* requires higher nutrient concentrations to grow at the same rate as at warmer temperatures. For *Aulacoseira granulata,* the peak growth occurred between 20 to

30°C (Coles & Jones, 2000), in tropical ecosystems, a temperature arround 21°C has been identfied as the optimum for Aulacoseira tenella, while for *A. brasiliensis*, warmer conditions (30.8 °C) are identified (Bicudo et al., 2016). Sub-optimal temperature shapes nutrient demand and shifts abundance and growth in algal populations (Staehr & Sand-Jensen, 2006).

2.4.4 Conclusions

The vertical distribution of *A. pusilla* in these alpine lakes showed different patterns, and when coupled with the experimental observations, indicate that the occurrence and density of this species is controlled by light and its interactions with nutrients and temperature. The fact that our study occurred under stratified conditions complemented the ecological understanding of this taxon, filling gaps in our understanding of seasonal patterns. The study of *A. pusilla* between mixing and stratification revealed the role of light and nutrients and shows the path that future research must address. Having more information in this regard will clarify the roles of different variables and address apparent contradictions stated by others (Denys et al., 2003; Akihiro Tuji, 2015; Williams et al., 2016a) about the environments that favor *Aulacoseira*. A deeper understanding of the ecology of this taxon will not only address gaps in autecological knowledge but it will also improve the prediction power of the taxon for paleolimnological studies.

3 CHAPTER 3. DECIPHERING LINKS BETWEEN DIATOM TAXA IN THE *AULACOSEIRA DISTANS* COMPLEX TO AIR TEMPERATURE AND SNOWPACK TRENDS OVER THE PAST 2,000 YEARS IN LAKES OF THE GREATER YELLOWSTONE ECOSYSTEM

3.1 Abstract

Fossil records of various *Aulacoseira* taxa from sediments are often used to infer thermal-induced changes in lake stability, based on blooms during wind- induced turbulence and lake isothermal conditions in spring and autumn.

Difficulties with taxon identification are a source of misinterpretation of fossil records. Recent work with the *A. distans* complex suggests ecological differences among these taxa, which have implications for accurate inferences about light and water column conditions from the distribution of these species. Using refined taxonomy in the analysis of lake sediment cores from the Greater Yellowstone Area (GYA), we assessed whether better resolution of these taxa would provide new insights about paleoclimate inferences of air temperature and snowpack at high elevations. We determined sedimentary diatom profiles from two lakes in this region, Beauty and Kersey, and linked them with climatic periods during the late Holocene using tree-ring based reconstructions of air temperature and snow water equivalent (SWE) from the GYA. Using Principal Component Analysis and Redundancy Analysis, we examined associations among the diatom assemblages, and identified the links between *Aulacoseira* taxa with changes in SWE and air temperature. We found three clusters of diatom assemblages across the Beauty Lake core, with the oldest cluster occurring during cooler conditions, a second cluster generally aligning with the Medieval Climate Anomaly, and the third cluster spanning the Little Ice Age and recent warming. In particular, *A. pusilla* peaked in

times associated with warmer conditions and was linked with low SWE values. A similar trend for this taxon was observed in Kersey Lake, whereas the timing of peaks in *A. alpigena* differed between the lakes and suggested lake-specific responses to the same climate forcing. The application of refined *Aulacoseira* taxonomy to these two cores, coupled with tree-ring based climate inferences, revealed the responsive nature of this group of diatoms to climate-driven lake ecosystem changes and suggests greater potential for the use of this genus in extending SWE inferences back further in time.

3.2 Introduction

Diatom taxa in the genus *Aulacoseira* Thwaites, 1848, are often used in paleolimnological reconstructions of climate (e.g., (Ruhland *et al.*, 2008; Wang *et al.*, 2008; Stone *et al.*, 2016), supported by contemporary studies that indicate associations between its occurrence and thermal-induced changes in lake stability (Lund, 1959; Reynolds *et al.*, 1986; Interlandi *et al.*, 1999). The relationship between *Aulacoseira* abundance and water column stability is based on observations of blooms during wind-induced turbulence and lake isothermal conditions in spring and autumn (Lund, 1959, 1971). This relationship has been useful to identify warm periods like the Bølling-Allerød, based on low relative abundances of *A. granulata,* and *A. ambigua*, as well as cooler conditions such as those during the Younger Dryas and Little Ice Age, sustained on changes in relative abundances of *A.granulata, A. lirata and A. perglabra* (Rühland and Smol, 2005; Wang *et al.*, 2008; Hobbs *et al.*, 2010; Johnson *et al.*, 2013).

The strength of diatom-based paleoclimate inferences rests on the quality of taxonomic resolution and ecological knowledge. As with many diatom taxa used in paleolimnological studies, taxonomic issues with species in the *Aulacoseira* genus are a source of misinterpretation of the records (English and Potapova, 2009).

Consequently, there are contradictions in fossil-based environmental interpretations and autecological gaps as well. *Aulacoseira* taxonomy suggests that *A. subborealis var subbarctica*, *A. subarctica* f. *subborealis*, *A. alpigena*, *A. laevissima*, *A. distans*, *A. nivalis, A. nivaloides*, *A. pusilla*, and *A.nygaardii* form the *A. distans* complex (Denys *et al.*, 2003). Nevertheless, research on individual taxa suggests ecological differences among these taxa that have implications for accurate inferences about light and water column conditions from the distribution of these species. The observation that turbulence stimulates *Aulacoseira* blooms suggests that species in this genus have low light requirements and spring and/or autumn occurrences during lake turnover periods (Lund, 1971). However, research has revealed greater spatial and temporal differences in the distributions of these species. *A. subborealis* is usually found in turbid shallow or deep ecosystems during the autumn, while *A. subarctica* was reported during the springtime, associated with large and turbulent lakes (Denys *et al.*, 2003). Paleolimnological records of *A. alpigena* in meso-humic oligotrophic lakes suggest it blooms from the summer to the beginning of the winter (Ilmavirta and Kotimaa, 1974; Miettinen, 2005). This taxon was also found in coexistence with *A. lirata* in deep alpine and subalpine lakes, where they are used as indicators of prolonged lake turnover and/or deep summer thermoclines driven by increased precipitation and changes in snowfall (Stone *et al.*, 2016). Records of *A. subarctica* in Kamchatka have been used to

identify warm summertime conditions in the late Holocene, but peaks of the same species in younger sediments (last 300 years) of the same lake also coincided with cool summer conditions; this apparent discrepancy suggests that the link for the taxon is more complex than solely temperature fluctuation and opens the possibility of links with precipitation. Precipitation affects turnover period through ice-cover and snow accumulation (Solovieva *et al.*, 2015). Clearly, improved resolution of the taxonomy and ecology of *Aulacoseira* taxa is warranted to improve paleolimnological inferences.

Research on the factors driving the growth and distribution of *A. pusilla* in two high elevation lakes of the Greater Yellowstone Area (GYA) has contributed to deciphering the ecology of this species (Pedraza-Garzón & Saros, in review). The vertical distribution of the taxon is not controlled by turbulence but by light. The link with light is complex and affected by nutrient availability. In experiments, *A. pusilla* populations from Kersey Lake, a nutrient depleted and moderately transparent system, fared better under high light incubations in surface waters with nutrient addition, while they were constrained at deeper depths. In contrast, the population of Beauty Lake, a highly transparent system, had recently experienced a spring pulse of nutrients. These populations fared equally well in shallow and moderately deep water. The vertical distributions of this taxon in Kersey and Beauty lakes reflected these experimental results. In Beauty Lake, early-summer populations were concentrated between mid to deep water, while in Kersey Lake, mid- summer populations peaked in surface waters. Understanding of these complex relationships between light and nutrients provides new insights that can be applied to paleoclimate inferences.

Refining diatom-based paleoclimate reconstructions from alpine lakes provides the

potential to extract more information about snowpack and seasonal variability at high elevations. Winter precipitation (snowfall) regulates alpine lake phenology and hydrological seasonal processes downstream. Thus, snowpack is a critical factor in mountain ecosystems (Adrian *et al.*, 2009; Moser *et al.*, 2019; Preston *et al.*, 2016; Livingstone, 1997). Changes in the amount of snow are quantified by snow water equivalent (SWE) which has been reconstructed at annual to multi-decadal resolution using tree rings (Pederson *et al.*, 2011). However, the use of this proxy is limited to the past ~1500 years, as well as elevations below treeline. Developing additional proxies for snowpack that extend the temporal and spatial resolution possible in alpine regions would strengthen our understanding of variability in winter precipitation and spring temperatures over time. Clarifying links between various *Aulacoseira* taxa and SWE holds promise, given the influence of water column structure and light on these species. Cooler conditions delay snow thawing and ice-off timing (Preston *et al.*, 2016; Smits *et al.*, 2020), affecting lake thermal structure and light attenuation.

With improved taxonomic resolution of *Aulacoseira* taxa and new autecological information for a key species, *A. pusilla*, we examined 2,000-year sedimentary diatom profiles from two high-elevation lakes in the GYA that contained a diverse array of *Aulacoseira* taxa. Our aim was to assess whether applying finer taxonomic resolution of this group yielded greater insights into links between climate and lake response, and hence could improve and refine paleoclimate inference tools from lake sediments. Specifically, we applied updated *Aulacoseira* taxonomy to the sedimentary diatom profiles of these two lakes and compared these to trends in tree-ring inferred air temperatures and snowpack from the region. Air temperature and snowpack are linked to the timing of ice off in high elevation lakes (Preston

et al. 2016), which in turn can affect lake turnover and thermal stratification conditions and thus play a role in shaping diatom community structure (Saros *et al.*, 2012; Warner *et al.*, 2018).

3.2.1 Methods Study site

Beauty Lake (44.97°N, 109.57°W) and Kersey Lake (45.03°N, 109.84°W) were selected based on previous reports revealing the presence of various *Aulacoseira* taxa in these lakes (Saros *et al.*, 2003; Stone *et al.*, 2016). Beauty and Kersey lakes are deep (>15 m), small (surface area < 1 km^2), oligotrophic, circumneutral (pH 6-7.6), and clear (1% attenuation depth for photosynthetically active radiation (Z1%PAR) of at least 10 m).

The lakes are located in the Beartooth Mountains, part of the Absaroka Beartooth Wilderness of Montana and Wyoming in the Central US Rocky Mountains. Watershed bedrock consists of Archean Gneiss and accessory metamorphic rocks (Love and Christiansen, 1985). The lakes are situated in slow weathering, U- shaped glacial valleys, with treeline between 2750 to 3000 m above sea level (Saros *et al.*, 2003). The area experiences continental climate, with the lowest but longest snow accumulation (peaking in April) for the Western US, with the snowpack ablation season around June (Trujillo and Molotch, 2014), and the lake ice-free season from early July to October.

Climatic studies in the central US Rocky Mountain suggested the area experienced minimum impact by climate change based on simulations using late 19^{th} to early 20^{th} century data (Knowles *et al.*, 2006; Mote, 2006; Scalzitti *et al.*, 2016). However, drought and snowpack reconstructions since ~1200 CE suggest that alpine zones in the area are susceptible to change due to precipitation shifts (Martin *et al.*,

2020; Pederson *et al.*, 2011). Upper Yellowstone Area snowpack reconstructions summarized changes as a shift from dry to wet conditions from the first to the second millenium (Shuman *et al.*, 2018). The causes for the changes, according to annual precipitation records from Yellowstone National Park region, are linked to multidecadal oscillations associated with the interplay between the Pacific Decadal oscillation (PDO) and the Atlantic Multidecadal Oscillation (AMO) (Gray *et al.*, 2007). Hence, the North Pacific and Atlantic ocean temperature oscillations were responsible for severe droughts in the area, such as the 1930s (PDO+ and AMO+) and 1950s droughts (AMO- and PDO-), the extremely dry years of the late 16^{th} century (AMO- and PDO+), as well as the 15^{th} and 20^{th} century pluvial anomalies (Fig 1) (Hidalgo, 2004). During the 13^{th} century there was exceptional precipitation, but sustained drought periods too. The anomalies of the Little Ice Age (LIA) were lower than those in the Medieval Climate Anomaly (MCA), and the change to wet conditions in the transition to the 20^{th} century correspond to the increase in the strength of La Niña phenomenon (**Figure 3.1**) (Martin *et al.*, 2019).

3.2.2 Paleolimnological analyses

Sediment cores were collected from Beauty Lake in 2007 and Kersey Lake in 2014 using a piston corer. The cores were extruded in the field in 0.5 cm-increments.

Given the lack of wood or pine needles in the core, radiocarbon dating was conducted on concentrated pollen isolations. Pollen was isolated by centrifugation using POLY-GEE sodium polytungstate (density 2.8) powder (GEOLiquids Inc.).

Samples were dated at the Lawrence Livermore Center for Accelerator Mass Spectrometry (CAMS). Age models and error estimates were created using R package, BChron (version 2.12.2). All radiocarbon dates were calibrated to calendar years using CALIB 5.0 (M. Stuiver, PJ Reimer and R. Reimer) and will be referred to herein as calibrated ages in years BP.

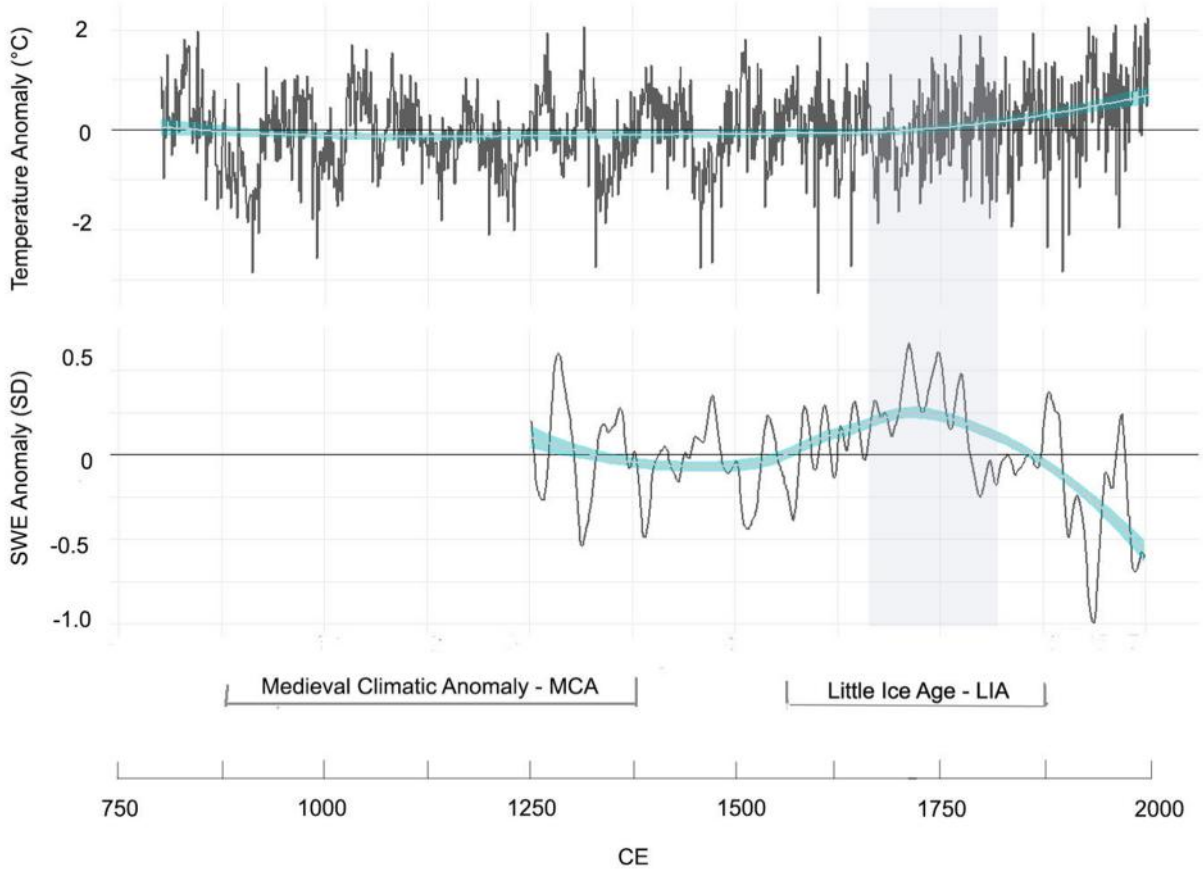


Figure 3.1 Temperature and Snow Water Equivalent (SWE) anomaly reconstruction for the Upper Missouri River in the Greater Yellowstone Area.

Gray vertical bar corresponds to the Maximum Holocene Glacier advance. Temperature anomalies graph shows the 20-year cubic smooth spline. Graph adapted from (Martin et al., 2019; Pederson et al., 2011)

Diatom samples were prepared digesting 0.5gr of each sample with 30% hydrogen peroxide. For the permanent slides we used Naphrax resin for mounting the diatoms. To ensure 95% of confidence interval, a minimum of 300 frustules was counted at 1000X magnification, using an Olympus BX51 microscope with phase contrast. The taxonomic identification was based primarily on multiple resources (Krammer and Lange-Bertalot, 1986; Krammer, 1991; Siver and Kling, 1997; Camburn and Charles, 2000; Buczkó *et al.*, 2010; Wetzel *et al.*, 2015).

3.2.3 Climate reconstructions

Climate reconstructions for the area spanning the late Holocene were based on tree-ring inferred air temperature (Martin *et al.*, 2019), and snowpack measured as SWE was presented as 20-year cubic smoothing spline of the standard deviation of composite April 1 SWE (https://www.ncdc.noaa.gov/paleo/study/26831) (Pederson *et al.*, 2011). We used the SWE standard deviation because in mountainous areas snow presents large spatial variability and the readings come from flat clear locations that can report higher values than their surroundings (Raleigh and Lundquist, 2012). The air temperature reconstructions spanned from 800 CE to 2005 CE, while the SWE data covered the time between 1252 to 1999 CE. Anomalies are expressed in relation to the mean of that period. Air temperature and SWE anomalies permitted us to identify generally warm and cold periods, as well as ablation- thaw trends of the time covered.

3.2.4 Statistical analyses

Relative abundances of the diatom taxa in the sedimentary profiles of each lake were used for statistical analyses using RStudio version 1.3.1056 (Rstudio Team, 2020).

To understand the composition and changes in diatom assemblages, we constructed stratigraphic plots using the vegan package for R (Oksanen *et al.*, 2019), and included only the abundant species (>5% of the relative abundance). Possible changes in the composition were detected by chronological clustering using Bray Curtis distance and compared with broken stick models. These analyses were conducted in the packages rioja (Juggins, 2017), and vegan (Oksanen *et al.*, 2019), using the functions chclust, vegdists, and bstick, respectively. The groups identified by this technique were linked to the age model for Beauty Lake sediments and the climatic periods during the Holocene (Pederson *et al.*, 2011; Shuman *et al.*, 2018). Since Kersey Lake sediments still do not have an age model, clusters were associated to depths of the sediment core.

Principal Component Analysis (PCA) was used to examine associations among the diatom assemblages identified by cluster analyses. This method used centered, non-scaled, square root relative abundance, and was run with the function rda (vegan package) and plotted with the autoplot function in the package ggfortify (Tang *et al.*, 2016; Oksanen *et al.*, 2019). Redundancy Analysis (RDA) (function rda, library vegan) was used to identify species and groups of assemblages that were associated with particular trends in tree-ring inferred climate parameters. The climate data used for this analysis covered the 1252-1999 CE span that corresponds to the time with both SWE and air temperature data.

3.3 Results

3.3.1 Updated *Aulacoseira* taxonomy

Our re-examination of the Beauty Lake core revealed a suite of *Aulacoseira* taxa spanning the core (**Figure 3.2**). The most abundant were *Aulacoseira alpigena* (Grunow) Krammer 1991, *Aulacoseira lirata* (Ehrenberg) R.Ross 1986, *Aulacoseira pusilla* (F.Meister) A.Tuji & A.Houki 2004 and *Aulacoseira perglabra* var *florinae* (Camburn) E.Y.Haworth 1990. Among the species with low relative abundances we found *Aulacoseira ambigua* (Grunow) Simonsen 1979, *Aulacoseira perglabra* (Østrup) E.Y.Haworth 1990, *Aulacoseira distans* (Ehrenberg) Simonsen 1979, *Aulacoseira italica* (Ehrenberg) Simonsen 1979 and *Aulacoseira subarctica* (O.Müller) E.Y.Haworth 1990. Previous work on the Beauty Lake sediment core indicated two abundant *Aulacoseira* taxa, *A. lirata* and *A. alpigena* (Stone *et al.*, 2016), with the latter being moved to *A. pusilla* recently (Spaulding *et al.*, 2020).This was the first identification of sedimentary diatoms in a core from Kersey Lake so there is no comparison to prior work for this lake.

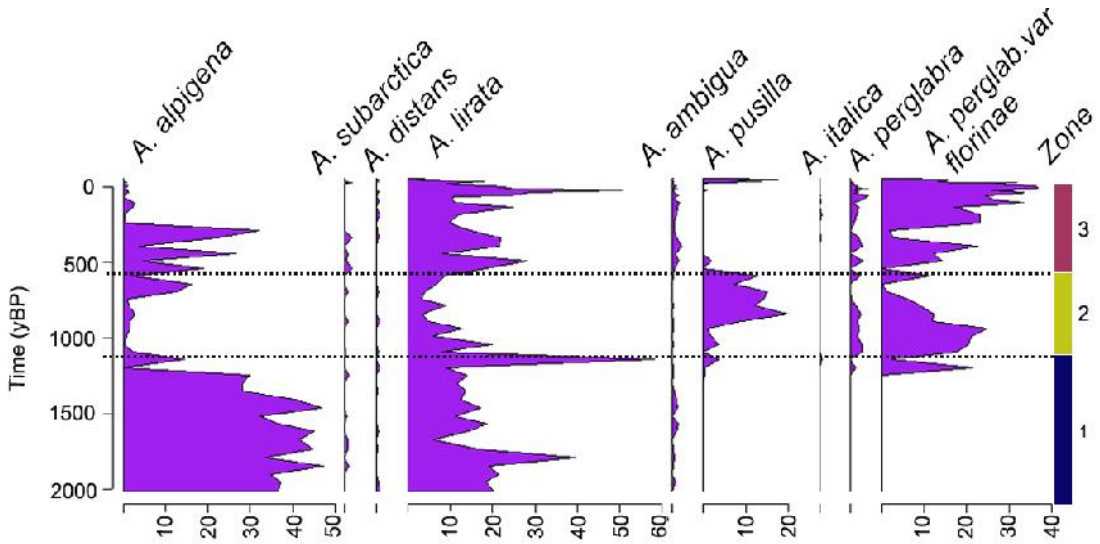


Figure 3.2 Percent relative abundances of Aulacoseira species in the Beauty Lake sediment core. Zone 1 2000-1116 y BP, Zone 2: 1116-566 y BP, Zone 3: 566- to -55 y BP.

For the Kersey Lake core (**Figure 3.3**), the diversity of *Aulacoseira* species was greater than in the Beauty Lake core. The most abundant species were *Aulacoseria granulata* (Ehrenberg) Simonsen 1979, *A. distans, A. alpigena*, and *A. pusilla*, while species such as *A. lirata* and *A. valida* that were abundant in the Beauty Lake core did not reach relative abundances greater than 5% in Kersey. Similar to the Beauty Lake sediment record, *A. ambigua* and *A. perglabra* were found at low relative abundances. Additional taxa present in low relative abundances in Kersey Lake were *Aulacoseira crenulata* (Ehrenb.) Thwaites 1848, *Aulacoseira crassipunctata* Krammer 1991, *A. perglabra*, *A*. cf. *preglabra var florinae*, *Aulacoseira islandica* (O.Müller) Simonsen 1979, *Aulacoseira nygaardii* (Camburn) Camburn & Charles 2000, *A.* cf. *italica*, *Aulacoseira muzzanensis* (F.Meister) Krammer 1991 and *Aulacoseira* cf. *tethera*.

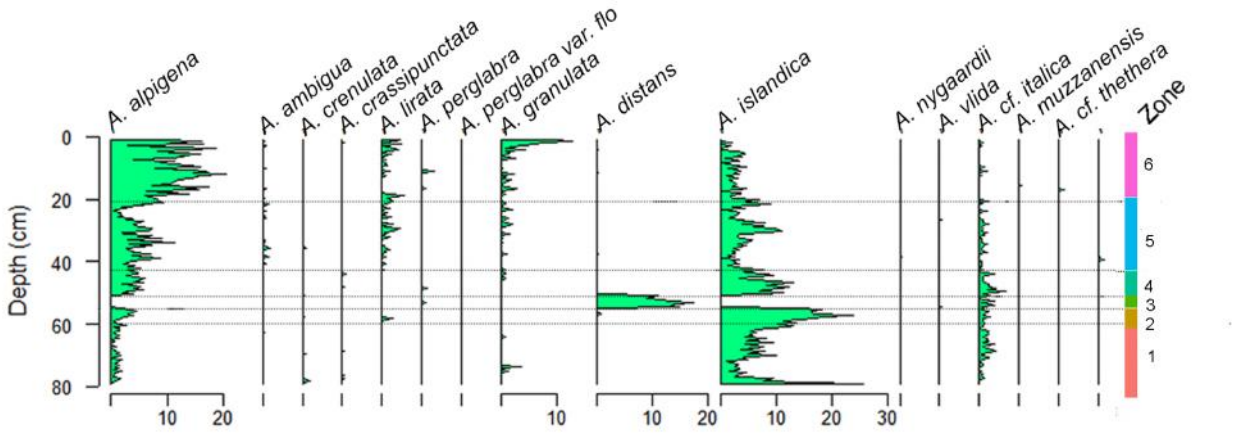


Figure 3.3 Percent relative abundances of Aulacoseira species in the Kersey Lake sediment core. The color scale and numbers at the right of the graph correspond to breaks in the structure of the assemblages according to Bray-Curtis analysis.

3.3.2 Diatom assemblages over time

In the Beauty Lake core (**Figure 3.4**), the most abundant taxa over the past 2,000 years were *A. alpigena* followed by *Discostella stelligera* (Cleve & Grunow) Houk & Klee 2004. Cluster analysis identified three groups. The first group (Z1) occurred from 2000 to 1116 years BP (50 BC to 834 CE), had the lowest number of species, and was dominated by *A. alpigena, D. stelligera*, *A. lirata*, *Pseudostaurosira brevistriata* (Grunow) D.M.Williams & Round 1988, and *Psammothidium marginulatum* (Grunow) Bukhtiyarova & Round 1996. The second group (Z2), spanning from 1116 to 566 years BP (834-1384 CE), showed a decrease in *A. alpigena*, and was dominated by *A. perglabra* var *florinae.* This was the cluster with the first peak in *A. pusilla*, occurring after 911 yBP. Tree-ring based reconstructions indicated that this cluster of diatom assemblages occurred during a period with temperatures below but closely around the average (Fig 1). SWE inferences were not available for this whole period; however, streamflow reconstructions for the area suggested that during this time frequent

precipitation fluctuations occurred that increased at the end of the term (Martin *et al.*, 2019) as well as many droughts that occurred since the 11th to 13th centuries. The last cluster (Z3) occurred from 566 to -55 yBP (1384 to 2005 CE). It showed an increase of diversity after 48 yBP, characterized by peaks in *A. pusilla,* cyclotelloids (*Lindavia, Cyclotella*), and small fragilaroids, and the increase in relative abundance of *A. perglabra var. florinae*. This period captured the cool trend prior the LIA that had negative SWE anomalies, the Little Ice Age with an increasing trend in SWE and the contemporary time where temperature increase and SWE decrease was registered.

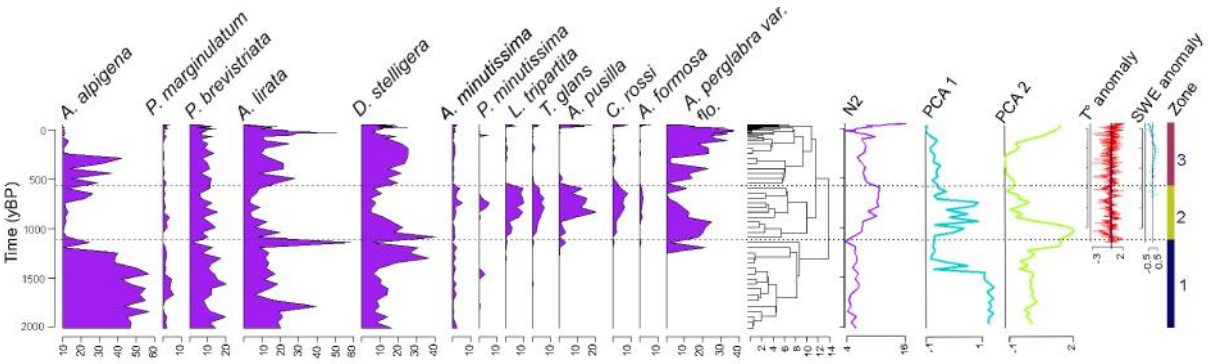


Figure 3.4 Vertical profile in the Beauty Lake sediment core.

Profile of dominant taxa (total relative abundance greater that 5%), Bray-Curtis dissimilarity clusters, Hill's N2, and axis 1 and 2 of principal component analysis (PCA) over time.

A.alpigena: Aulacoseira alpigena; P. marginulatum: Pseudostaurosira marginalatum; P. brevistriata: Pseudostaurosira brevistriata; A. lirata: Aulacoseira lirata; D. stelligera: Discostella stelligera; A. minutissima: Achnanthes minutissima; P. minutissima: Pseudostaurosira minutissima; L. tripartita: Lindavia tripartita; T. glans: Tetracyclus glans; A. pusilla: Aulacoseira pusilla; C. rossi: Cyclotella rossi; A. perglabra var. flo: Aulacoseira perglabra var. florinae

Tree-ring inferred air temperature and SWE anomalies are also indicated. Zones indicated at the right of the graph correspond to breaks in the structure of the assemblages according to Bray-Curtis analysis. Zone 1 2000-1116 y BP, Zone 2: 1116-566 y BP, Zone 3: 566 to -55 y BP.

PCA revealed that changes in the relative abundances of *Aulacoseira* taxa over the Beauty Lake core were important in differentiating the identified zones (**Figure 3.5**). PCA axis 1 explained 53% of the variance, and axis 2 explained 24%. Along PCA axis 1, assemblages in the oldest cluster spanning 2000 to 1116 y BP (50 BC to 834 CE) plotted opposite to those of the other two clusters. Assemblages in the clusters spanning from 1116 to 566 years BP (834 to 1384 CE) and 566 to -55 yBP (1834-2005 CE) separated out along PCA axis 2.

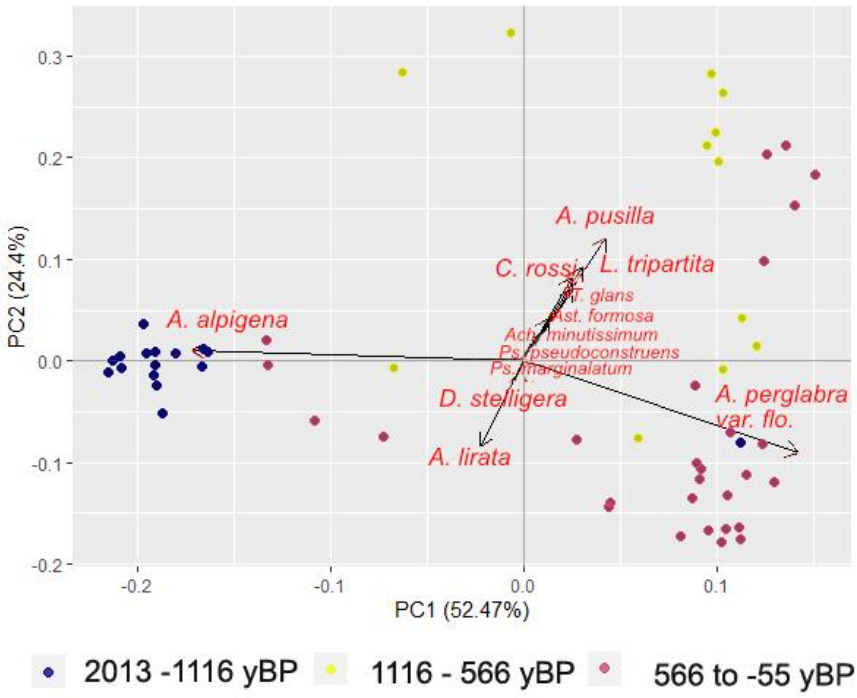


Figure 3.5 PCA biplot of the diatom assemblages in Beauty Lake from 2013 to -55 yBP. Colored circles correspond to the assemblages identified by the broken stick model analysis. Acronyms are the same as in figure 3.4

In the Kersey Lake core (**Figure 3.6**), the most abundant diatoms were *D. stelligera* followed by *Lindavia comensis* (Grunow) T.Nakov et al. 2015, *Lindavia bodanica* (Eulenstein ex Grunow) T.Nakov, Guillory, Julius, Theriot & Alverson 2015, *Staurosirella pinnata* (Ehrenberg) D.M.Williams & Round 1988, and *Pseudostaurosira parasitica* (W.Smith) E.Morales 2003. Cluster analysis identified six groups. The first group (Z1) occurred between the depths 80 to 60.5 cm. This section of the core was dominated by *L. bodanica* accompanied by small *Fragilaria sensu lato* taxa such as *S. pinnata* and *P. parasitica*. The second cluster (Z2) occurred between 60 to 55.5 cm with *L. bodanica* and *A. distans* dominating. Z3 occurred between 54.5 to 51 cm and was characterized by the occurrence of new species such as *A. granulata* along with *Cyclotella* sp, and small *Fragilaria sensu lato* (*Stauroneis smithi* var. *incisa* and *Staurosirella* cf. *pinnata*). Z4 (50.5-43.5 cm) was dominated by *L. bodanica* and *L. comensis*. The fifth cluster (43 to 20.5 cm) was characterized by *Discostella* and *Lindavia* species. At the top of the core (20 to 0 cm), Z6 was dominated by *D. stelligera* and high relative abundance of *Aulacoseira* species (*A. pusilla*, *A. alpigena*, and *A. distans*). This diatom assemblage showed the lowest value of effective number of species.

PCA axis 1 explained 49% of the variance in diatom assemblages along the Kersey Lake core with *L. bodanica* and *D. stelligera* at opposite ends of this axis (**Figure 3.7**). Assemblages with higher abundances of *D. stelligera* (Z6 and Z5) plotted opposite to those dominated by *L. bodanica* (Z1 and Z4). The assemblages of Z3 plotted distinctly from others, along PCA axis 2 (17% of variance) and were characterized by *Cyclotella* sp and *A. granulata*, and low relative abundance of *L. comensis* as well.

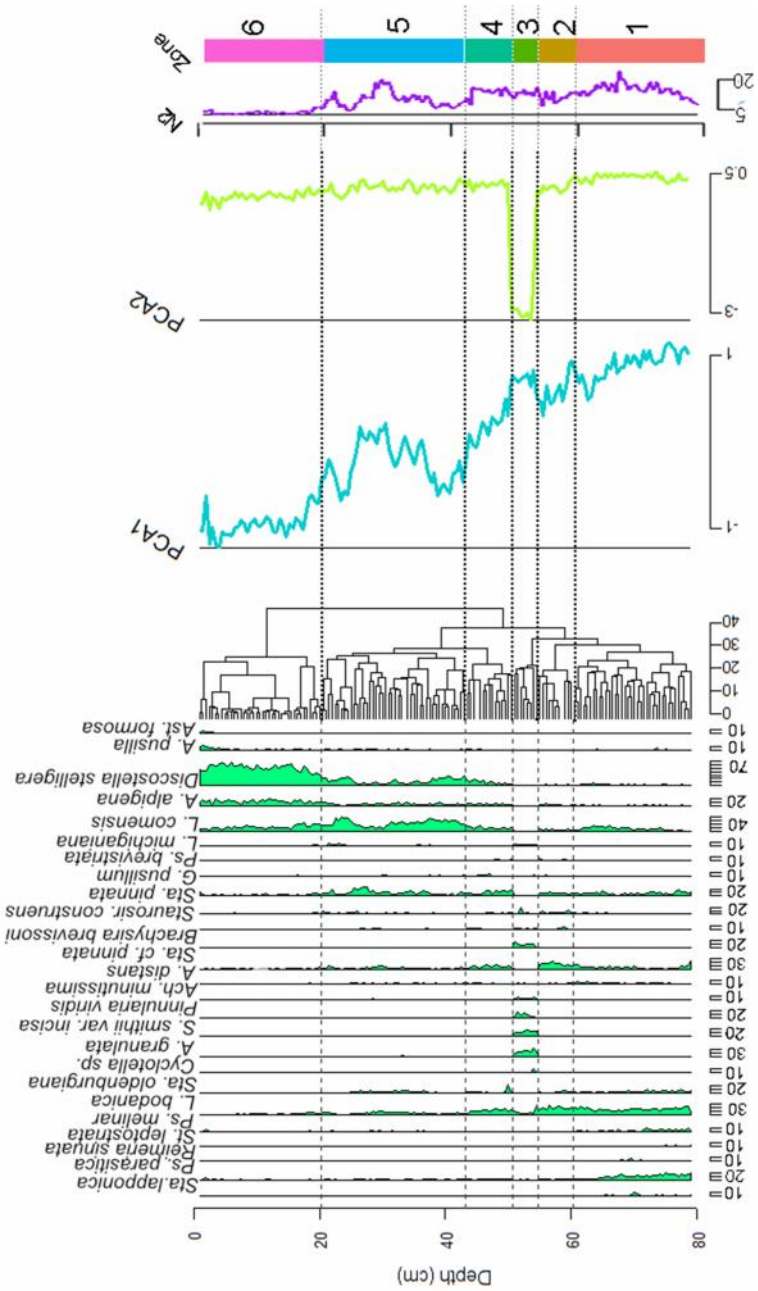


Figure 3.6 Vertical profile in the Kersey Lake sediment core.

Profile of dominant taxa (total relative abundance greater that 5%) in, Bray-Curtis dissimilarity clusters, PCA values for axis 1 and 2, and Hill's N2. Zones at the right of the graph correspond to breaks in the structure of the assemblages according to Bray-Curtis analysis

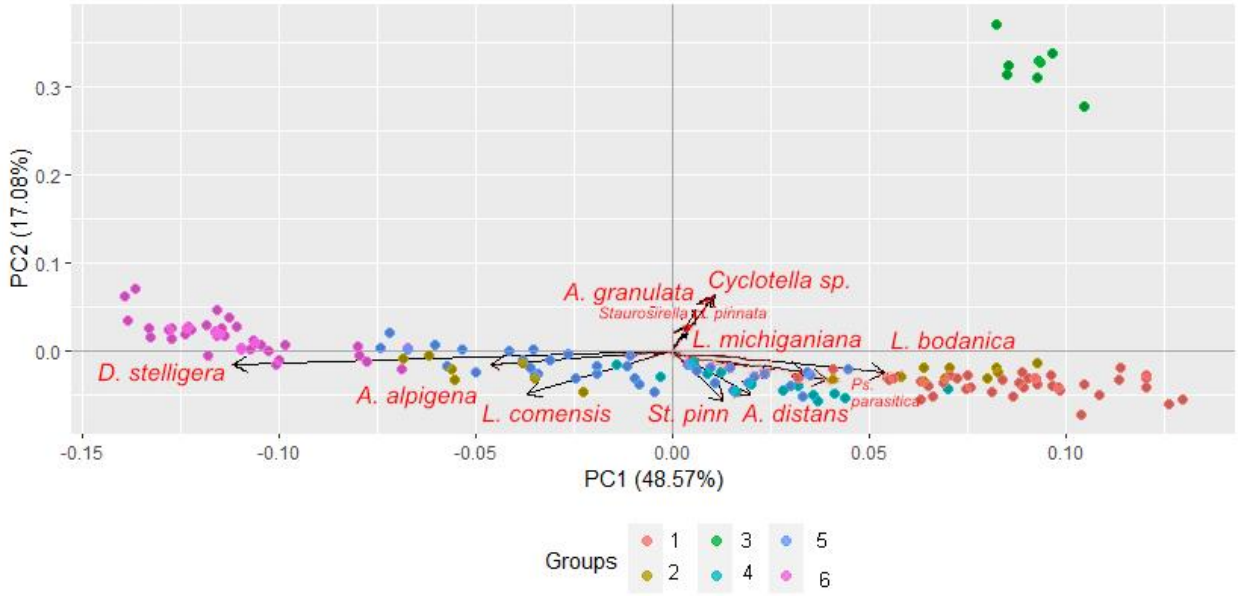


Figure 3.7 PCA Biplot of the diatom assemblages in Kersey Lake.

Colored circles correspond to the assemblages identified by the broken stick model analysis. D.stelligera: Discostella stelligera; L. comensis: Lindavia comensis; St. Pinn: Staurosirella pinnata; A. distans: Aulacoseira distans; Ps. Parasítica: Pseudostaurosira parasítica; L. bodanica: Lindavia bodanica; L. michiganiana: Lindavia michiganiana; A. granulata: Aulacoseira granulata

3.3.3 Climate linkages with *Aulacoseira*

The RDA analysis with tree-ring inferred air temperature and SWE values from 691 to -45 y BP showed that the environmental factors were responsible for 7% of the variability, and unconstrained factors explained 93% of the variation in *Aulacoseira* in Beauty Lake (**Figure 3.8**). The first RDA axis represented 91% of the constrained variance, it was defined by SWE. Air temperature guided the ordination on the second axis. Higher relative abundances of species such as *A. pusilla, A. per. var florinae* and *A. lirata* were associated with lower tree-ring inferred SWE, while *A. perglabra* and *A. italica* plotted at the high side of the SWE vector.

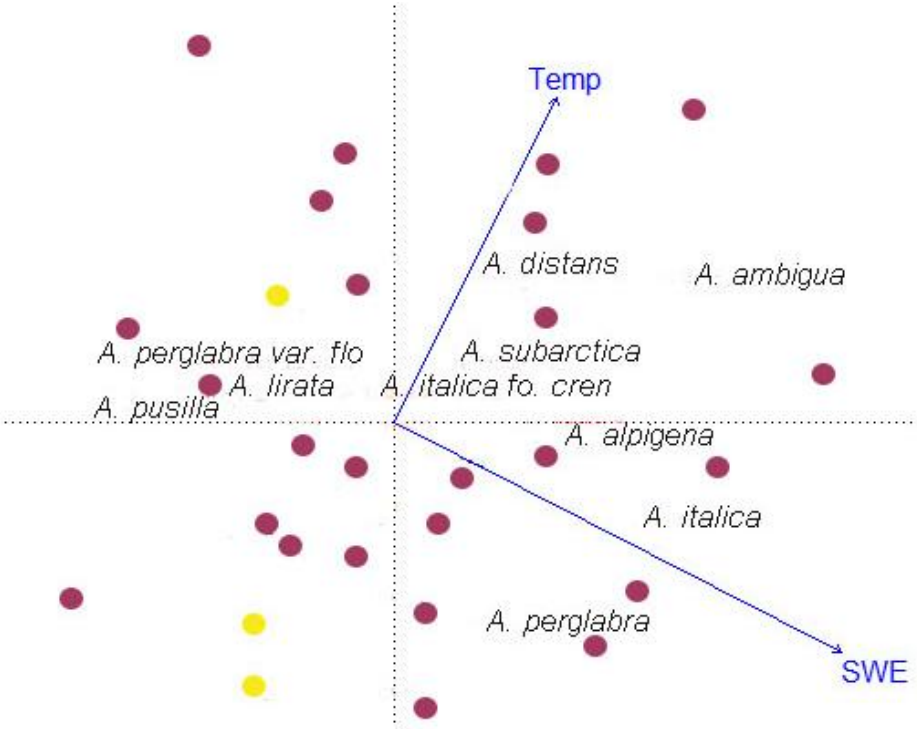


Figure 3.8 Redundancy analysis (RDA) of Aulacoseira taxa in Beauty Lake sediments and tree-ring inferred air temperatures and Snow Water Equivalent (SWE).

A.alpigena: Aulacoseira alpigena; A. pusilla: Aulacoseira pusilla; C. rossi: Cyclotella rossi; A. perglabra var. flo: Aulacoseira perglabra var. Florinae

3.3.4 Discussion

The use of updated taxonomy for the genus *Aulacoseira* revealed that Beauty and Kersey lakes have a rich record of *Aulacoseira* taxa, and the variations in their relative abundances in these sediment records were important to delineating clusters over time. The diatom assemblages observed in Beauty Lake over the last 2000 years showed that the lake is sensitive to climatic fluctuation, with the zones identified in the core corresponding with

climatic events in the area. While tree-ring based climate reconstructions are unavailable for the GYA during diatom Z1, climate inferences from other sources covering this time indicate that the climate was cold. Reconstructions suggest low solar irradiation and reduced sea surface temperature that caused frequent and prolonged El Niño events (Conroy *et al.*, 2008). The dominance of *A. alpigena* and generally lower diversity of diatoms characterized this zone. The sediments of diatom Z2 covers the warm conditions of the MCA. This time was summarized as a dry-wet-dry swing (Martin *et al.*, 2019) that was a product of high solar activity and El Niño like conditions (Conroy *et al.*, 2008; Yan *et al.*, 2011). These conditions would induce glacial retreat, while warmer and drier conditions caused the mega-droughts (McWethy *et al.*, 2010). The dominance of *A. pusilla* and *A. perglabra var. florinae* as well as the diversification of diatoms distinguished Z2 from Z1. Finally, the last zone of the core covers the time with the most variable conditions, from the LIA to the warmer, drier conditions of the contemporary time. During the LIA, cooler conditions characterized the climate as well as a dry-wet-dry swing. Although this zone captured the time with the most extreme precipitation and glacier advance (Helama *et al.*, 2017; Yan *et al.*, 2011), rising temperatures in the 20th century coincided with the lowest SWE values inferred.

Distributions of species in the *A. distans* complex are often related to turbid waters driven by climate cooling trends (Miettinen, 2005; Ilmavirta and Kotimaa, 1974; Koinig *et al.*, 1998; Dalton *et al.*, 2018; Schroeder *et al.*, 2016; Anderson *et al.*, 1996; Tsukada *et al.*, 2006). However, the use of refined taxonomy allowed us to better understand links between different *Aulacoseira* taxa and climate. High relative abundances of *A. alpigena,* used as an indicator of deep mixing and cooler climate (Stone *et al.*, 2016), were also found in our study

during cold spans, but they were also associated with moderate SWE values. These results do not contradict the inference about lake temperature and mixing (Stone *et al.*, 2016), and suggest that the occurrence of the taxon may be regulated in part by processes controlled by snow accumulation. Snowpack modulates the energy in lakes via the effect of insulation and limiting heat exchange (Smits *et al.*, 2020).

Thus, under high snow conditions, the melting action of radiation on snowpack and ice and the disturbing activity of wind is delayed, taking more time to induce ice- free conditions (Livingstone, 1997). Therefore, the deep mixing required for *A. alpigena* blooms can occur when the lake experiences prolonged spring.

In contrast, our finding of links between *A. pusilla, A. lirata,* and *A. perglabra var florinae* with low SWE suggests that these species could have high light requirements and are favored under higher light conditions. Open water periods or the thinning of ice and snow layer affect the light properties underwater and permit light to penetrate deeper in the water column (Jewson *et al.*, 2009; Lotter and Bigler, 2000). Previous studies on *A. pusilla* indicated that this taxon benefits from high light access (Pedraza-Garzón & Saros- in review). Thus, as low SWE permits light irradiance to reach deeper in the water column, it can stimulate the growth of the taxon. Similar results were reported in sediments from Meander Creek Reservoir in Ohio, where *A. pusilla* peaked since the 1980s when changes in the watershed led to increasing water transparency (Schroeder *et al.*, 2016). A study of boreal lake sediments noted that *A. perglabra* var *florinae* and *A. lirata* scarced in the 17th century, during the lowest temperatures (Anderson *et al.*, 1996). Our results suggest a stronger link with SWE than temperature for these taxa, as it was also reported for *A. subarctica* in

Holocene sediments from a coastal lake in the Kamchatka Peninsula (Solovieva *et al.*, 2015). Nevertheless, the contrasting distribution of *A. lirata* and *A*. *perglabra* var. f*lorinae* in the PCA suggest that other aspects not part of this analysis could cause the observed distributions.

In general, it should be noted that without tree-ring based climate inferences available for the whole record of diatom assemblages, this limited our ability to relate temperature and SWE to all of the shifts in diatom metrics. A broader set of values with both diatom data and climate inferences may strengthen the findings.

Additional inferences can be made by comparing the Beauty *Aulacoseira* record to the relative abundances of other diatom taxa in the record. Peaks of *A. pusilla* coincided with blooms of *Lindavia tripartita* (H.Håkansson) T.Nakov et al. 2015 and *Lindavia rossi* (Håk.) Nakov et al. 2015, usually grouped in a complex. These taxa have been associated with long ice-free periods and thermal-induced stability in lakes (Lotter and Bigler, 2000; Saros *et al.*, 2012), thus the co-occurrence with these species strengthens the inference between *A. pusilla* and changes induced by earlier ice thawing. A similar trend for *L. comensis* was observed in Hidden Lake, an alpine lake also in the GYA, which increased after the LIA (~1850CE) (Saros *et al.*, 2012).

Some differences in the timing of taxon peaks between Kersey and Beauty lakes suggest that the same climate forcing may have affected these two lakes differently. In both lakes, relative abundances of *A. pusilla* peaked in recent decades, which are relatively warm and dry; the species also peaked in Beauty Lake during the MCA. This taxon fares well under higher light conditions (Chapter 1); with the lower water clarity of Kersey compared to Beauty, this may suggest that conditions leading to a higher light environment (less snow,

earlier ice out) are more severe in recent decades compared to the MCA. Peaks in the relative abundances of *A. alpigena* also differed between these lakes, with highest relative abundances in Beauty Lake during a cooler period versus in Kersey Lake during the most recent period, which was warmer and drier. It is currently unclear what drove that difference in this species; further autecological work with this taxon would be useful for deciphering these differences.

With part of the variation in *Aulacoseira* attributed to factors other than temperature and SWE and the contrasting diatom distribution observed between lakes, top-down control may have also been involved. Studies on the grazing control of algal community composition have demonstrated that herbivore consumption affects planktonic composition (Kagami et al., 2002; Wigdahl-Perry et al., 2017). The main zooplankton impact is due to size-selective filter grazing (Leavitt et al., 1989). Large zooplankton graze on large or colonial algae, while small herbivores predate on small algae (Infante & Litt, 1985). Hence, the dominance of diatoms such as *A. alpigena or A. lirata* and the low abundance of *D. stelligera* in Beauty Lake in the oldest sediments might suggest that Cladocerans like *Daphnia* may have grazed on *D. stelligera.* It could be explained due to its smaller size, letting prosper the larger diatom species. A similar grazing effect was suggested for Emerald Lake sediment records (Fischer et al., 2011). During glacier accretion, deep mixing and extended lake ice layer would have created favorable conditions for *Daphnia*, which benefit from dim conditions (Clarke, 1930). Under deeper light access conditions (like contemporary time or MCA), probably affecting *Daphnia* dominance, a change in the zooplankton community may have occurred. It would explain the proliferation of small-sized diatoms. It is possible that during those times, the feeding size spectrum changed.

Studies about the contemporary zooplankton community in these lakes and herbivores' sediment remains will clarify the magnitude of the effect of higher trophic levels on the diatom composition.

3.3.5 Conclusions

The use of refined *Aulacoseira* taxonomy revealed multiple shifts in diatom assemblages over these two lake sediment records and for Beauty Lake, provided insight into how this lake may have responded to climate variability over the last two millennia. Changes in the relative abundances of certain *Aulacoseira* taxa*,* including *A. pusilla,* aligned with changes in SWE and suggested links to changing ice out timing. *Aulacoseira* taxa variation in Beauty Lake indicated that the area is facing changes in precipitation and that changes in snowfall (SWE) regulate aquatic communities of high-elevation lakes in the GYA via thermal structure of water column. Differences in the timing of peaks in some species such as *A. alpigena* in Kersey and Beauty lakes underscores the need for further autecological studies of these taxa to refine our understanding of the links with environmental change. With prior research indicating that *Aulacoseira* responds to changes in light access, a deeper understanding of how this factor regulates the growth and abundance of these taxa will be helpful.

4 CHAPTER 4. APPLYING UPDATED *AULACOSEIRA* TAXONOMY TO LAKE SEDIMENT RECORDS OF THE US CENTRAL ROCKY MOUNTAINS: PREVALENCE OF *AULACOSEIRA PUSILLA*, COMPARISON TO SIMILAR TAXA, AND SCOPE OF REVISED RECORDS

4.1 Abstract

The genus *Aulacoseira* Thwaites 1848 is widely distributed and often used in environmental reconstructions, but taxonomic problems may hinder inferences from fossil records. The lack of clear differentiation associated with valve morphology of the *Aulacoseira distans* complex leads to problematic identification. Recent studies identified that *A. pusilla* (part of the *A. distans* complex) was a common member of the phytoplankton in two lakes of the Beartooth Mountains in the Greater Yellowstone Ecosystem. This taxon was previously lumped with *A. alpigena* in these lakes, raising questions about the temporal and spatial distribution of *A. pusilla* in the Beartooth Mountains lakes. We examined previous slides from seven high-elevation lakes in the Beartooth Mountains to assess the prevalence of *A. pusilla* and other similar taxa, including *A. alpigena*, *A. nivaloides,* and *A. perglabra* var *florinae*. The stratigraphic distribution of *Aulacoseira* species was analyzed to identify changes in the relative abundances and associations between species. We found that the distribution of *A. pusilla* was limited to two out of the seven lakes studied; this species bloomed during times associated with warm conditions, which differed from *A. alpigena* peaks. *A. alpigena* showed contrasting patterns in Kersey and Beauty lakes, ecosystems which vary in water transparency. *A. nivaloides* in Kersey Lake decreased in recent sediments suggesting that warmer conditions are less favorable for this taxon. Finally, *A. perglabra* var *florinae in Beauty Lake was associated with periods of lower snowpack. The use of refined taxonomy* revealed a rich composition of

Aulacoseira that can be used for further climate studies. The temporal distribution patterns of *A. pusilla* revealed that the success of this species may be strongly linked to reduced SWE, and limitations in its distribution present the opportunity to explore the reasons behind the distribution of this species in a small area.

4.2 Introduction

The genus *Aulacoseira* Thwaites 1848, widely distributed and the oldest freshwater diatom (Ambwani et al., 2003; Kociolek, 2018), is frequently found in lake sediment records. The ubiquitous nature of this group makes it suitable for environmental reconstruction (Manoylov et al., 2009). However, identification problems have generated contrasting results that affect the inferences derived from *Aulacoseira* peaks (Denys et al., 2003; Solovieva et al., 2015; Akihiro Tuji, 2015).

The problems associated with identification not only affect paleoecological interpretations, but also contribute to autecological misunderstanding about species (J English & Potapova, 2011; M. Potapova, 2010). Most of the taxonomic problems are generally associated with low mantle height species (Buczkó et al., 2010), in which complexes such as *Aulacoseira distans,* are the focus of conflicts (Buczkó et al., 2010; Siver & Kling, 1997). The lack of clear differentiation of characteristics associated with valve morphology, and the use of light microscopy to distinguish those traits, leads to problematic identification (Edgar, 2003).

The study of the *A. distans* complex is important for taxonomy because under this name many morphospecies such as *Aulacoseira pusilla* (F.Meister) A.Tuji & A.Houki 2004*, Aulacoseira subarctica* (O.Müller) E.Y.Haworth 1990*,* and *Aulacoseira alpigena* (Grunow) Krammer 1991 are usually reported. Despite advances, there is still need for work to define the differences among the species in this group (Buczkó et al., 2010; Denys et al., 2003; J English & Potapova, 2011; Siver & Kling, 1997).

Recently, Pedraza Garzón & Saros (in review) identified that *A. pusilla* was a common member of the phytoplankton in two lakes of the Beartooth Mountains in the Greater Yellowstone Ecosystem of the US Central Rocky Mountains; the species was previously lumped with *A. alpigena* in these lakes. Analysis of sediment records from two of these lakes revealed variable and informative patterns of change in the relative abundance of this taxon over the past 2000 years (Pedraza Garzón, Chapter 2). These results raised questions about how widespread *A. pusilla* is across lakes and how often it has been misidentified in paleolimnological records in the Beartooth region. Given the numerous paleolimnological studies in this region and the frequent occurrence of *Aulacoseira* taxa across these lakes (Hobbs et al., 2010; Saros et al., 2003; Slemmons et al., 2015; A. A. Spaulding et al., 2010; Stone et al., 2016b), this region serves as a model study site for exploring these questions.

In light of these recent results and taxonomic issues, our aim here is to clarify the temporal and spatial distribution of *A. pusilla* in the Beartooth Mountains lakes and to compare the morphology and ecology of this species to those of similar taxa or those mainly observed in the assemblages, namely *A. alpigena* (Grunow) Krammer 1991, *Aulacoseira nivaloides* (K.E.Camburn) J.English & M.Potapova 2009, and *Aulacoseira perglabra* var *florinae* (Camburn)

E.Y.Haworth 1990. We describe the latest taxonomy of this group. We reexamine the *Aulacoseira* taxa in sedimentary diatom profiles of seven lakes in the Beartooth Mountains to assess the extent to which *A. pusilla* was underrepresented in original counts. In two lakes with the richest array of *Aulacoseira* taxa, we assess whether the different *Aulacoseira* taxa tend to co-occur.

4.3 Methodology

4.3.1 Study Site

The study focused on seven high-elevation lakes in the Absaroka-Beartooth Wilderness Area in Montana and Wyoming. We analyzed sediment cores from Jasper, Albino, Beauty, Kersey, Emerald, Hidden, and Rock Island Lake, which are characterized as deep (>15m), oligotrophic, and circumneutral lakes, located between ~2400 to 3100 m.a.s.l (Table 1) (Saros et al., 2003). These lake cores were selected because previous research indicated that all of them had abundant *Aulacoseira* taxa.

4.3.2 Methods

Information about the collection and age models for these cores are already available for Emerald Lake (Fischer *et al.*, 2011), Beauty Lake (Stone *et al.*, 2016; Spaulding *et al.*, 2019), Jasper and Albino lakes ((Fischer et al., 2011; Hobbs et al., 2010; Slemmons et al., 2015; A. A. Spaulding et al., 2010; Stone et al., 2016b), and Kersey Lake (Chapter 2). Hidden and Rock Island lakes were sampled using a gravity corer and dated using ^{210}Pb chronology; the sedimentary diatom profiles for these two lakes were not previously published.

Previously prepared slides were examined based on taxonomy from multiple sources (Camburn & Charles, 2000; Krammer, 1991; Siver & Kling, 1997; Akihiro Tuji, 2015). For Albino and Jasper lakes, we focused on *A. alpigena* and *A. distans.* In Emerald Lake, we focused on *A. perglabra,* while in Hidden Lake, we checked *A. distans* and *A. distans* var *nivalis*, and in Beauty Lake we re-analyzed *A. alpigena*. The Rock Island Lake core was not analyzed before, but surface sediments had *Aulacoseira perglabra var. florinae*.

4.3.3 Statistical analyses

In Beauty and Kersey lakes, stratigraphic distribution analysis of *Aulacoseira* species in sediments was used to identify the changes in the abundance of the taxa using the vegan package for R (Oksanen et al., 2019). Associations between *Aulacoseira* species were identified by clusters and correlograms of spearman coefficient correlations using the function cor, in the library corrplot (Wei & Simko, 2017).

4.4 Results

The re-examination of *Aulacoseira* taxa in the sediment cores from Emerald, Hidden, Rock Island, Jasper and Albino lakes did not alter previous taxonomic identifications, hence we focus here on results from Beauty and Kersey lakes.

***Aulacoseira pusilla* (F.Meister) A.Tuji & A.Houki 2004**

Basionym: *Melosira pusilla* F.Meister

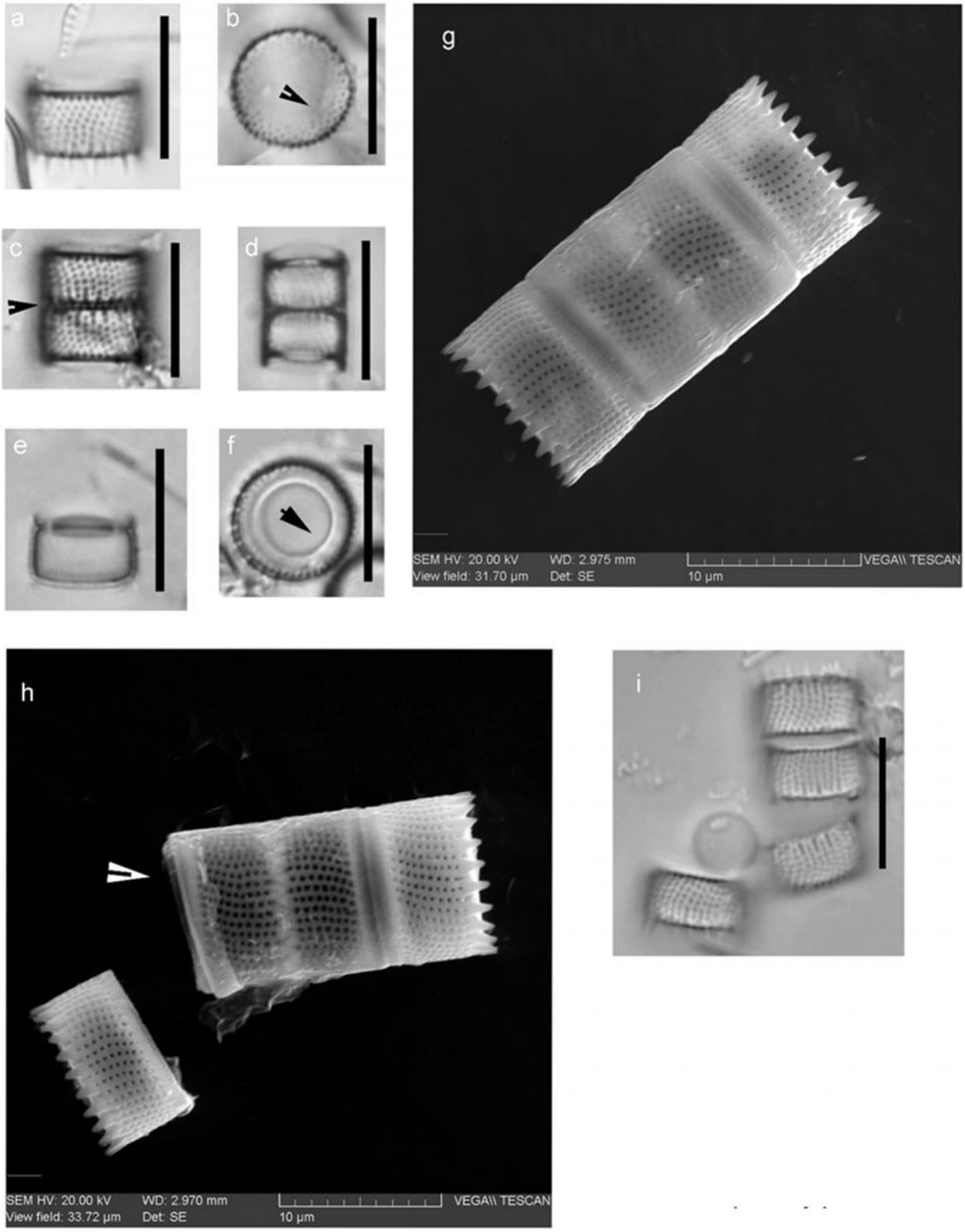
It has also been reported as *Aulacoseira subborealis* ((Nygaard) Denys, Muylaert and Krammer 2003, *Melosira italica var. subborealis* Nygaard, and *Aulacoseira subarctica* f. *subborealis* (Nygaard) Haworth (M. Potapova, 2010; M. G. Potapova et al., 2008).

This taxon shows a circular, isopolar frustule usually seen in girdle view. Valves 7-10 µm in diameter and 4-5µm height. Mantle height-diameter ratio:0.43. Valvar face flat with round areolae disperse around the central area. Mantle ornamented with curved striae of 7-9 areolae. Rim punctate product of short and robust marginal spines, striae density ~8/10µm. Two striae per spine (**Figure 4.1**a-k).

This taxon was only observed in Kersey and Beauty Lake sediment records; it was absent from the other five cores that were re-examined. In Beauty Lake sediments, the taxon was among the most abundant species. It was observed from 835-1384 CE, and at the top of the core, after 1983 CE. The maximum relative abundance (~20%) occurred during the Medieval Climate Anomaly (MCA) (~1039 to 1300 CE). It was absent during cold periods, prior the MCA and during the Little Ice Age (LIA) (**Figure 4.2**). In Kersey Lake sediments, the relative abundance

was lower than those observed in Beauty Lake sediments. It was present in the sediments at low relative abundances (<5%) throughout the core until the top, where it peaked (**Figure 4.3**).

In Beauty Lake sediments, moderate and negative associations (ρ=-0.50 to -0.75) were identified with *A. lirata* (Ehrenb.) R.Ross 1986 ($p < 0.001$) and *A. ambigua* (Grunow) Simonsen 1979 ($p< 0.001$), while associations were weak ($\rho<\pm0.50$) with the remaining *Aulacoseira* species (**Figure 4.4**). All associations with *A. pusilla* were weak *(°<±0.50)* in Kersey Lake, but were only significant with *A. alpigena* ($p <0.001$) and *A. lirata (p < 0.001).*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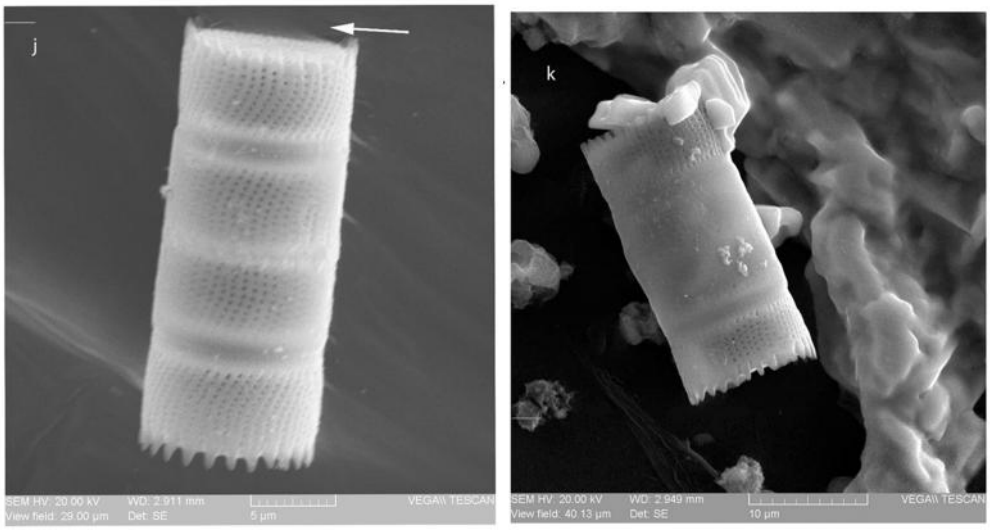

Figure 4.1 *Aulacoseira pusilla*.

a). Valve in girdle view showing long-curved-robust striae at the margin of the valve. b). Valve face. Arrow showing the central area sparcely punctate. c). Sister valves. Arrow pointing the link and the characteristics of the spines, d). Contour of sister valves, e). contour of single valve, f). Valvar view. Arrow indicates the ringsleist, g and h). SEM images of *A. pusilla* chains. Arrow shows the girdle band, i). Frustule and single valves. Scale bar in LM pictures corresponds to 10um. j). SEM Image of *A. pusilla* chain. Arrow showing the areolae present in the valve face. k). SEM image of *A. pusilla* chain wrapped with a mucillagus coating.

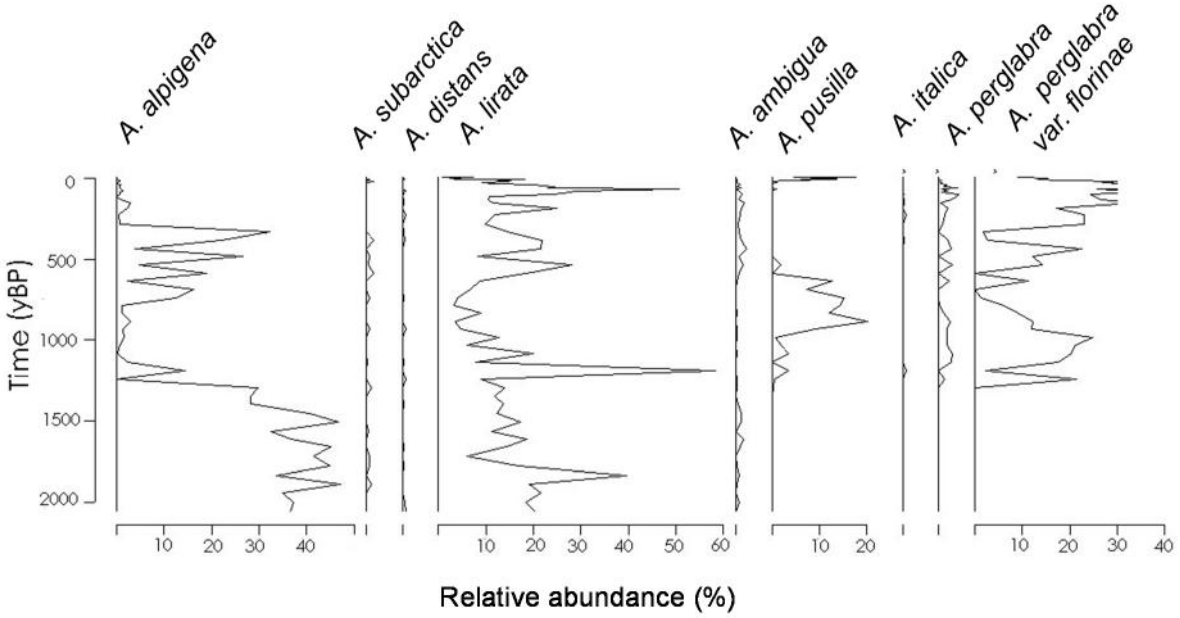

Figure 4.2 Percent relative abundances of *Aulacoseira* species in the sediment core profile of Beauty Lake.

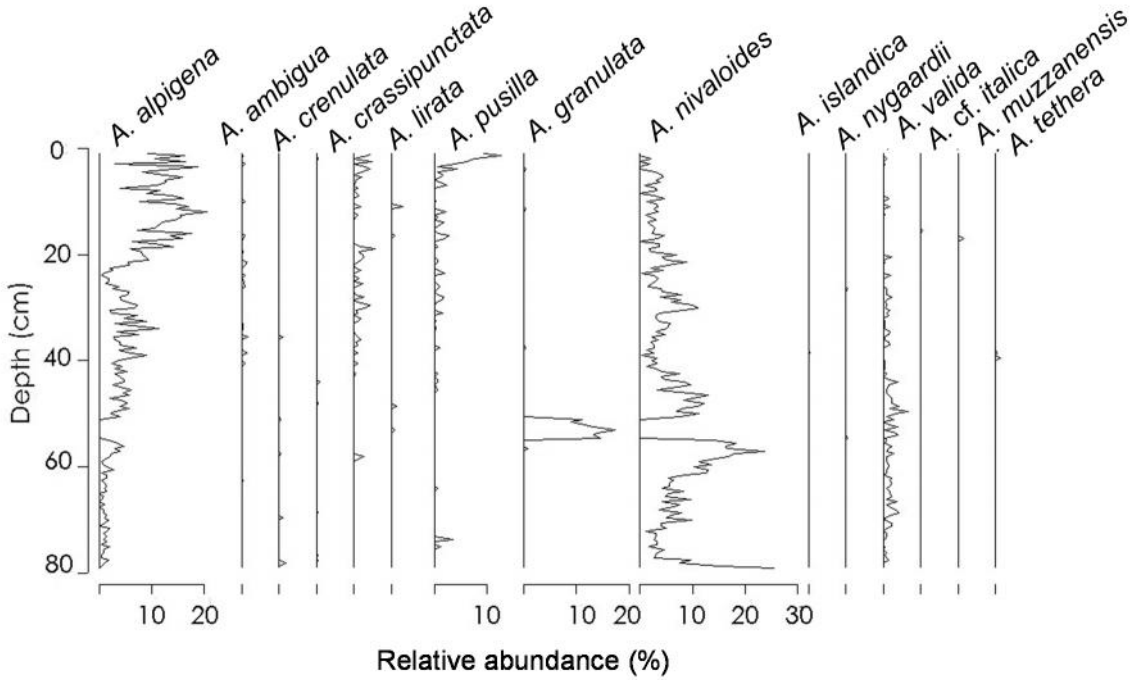

Figure 4.3 Percent relative abundances of *Aulacoseira* species in the sediment core profile of Kersey Lake.

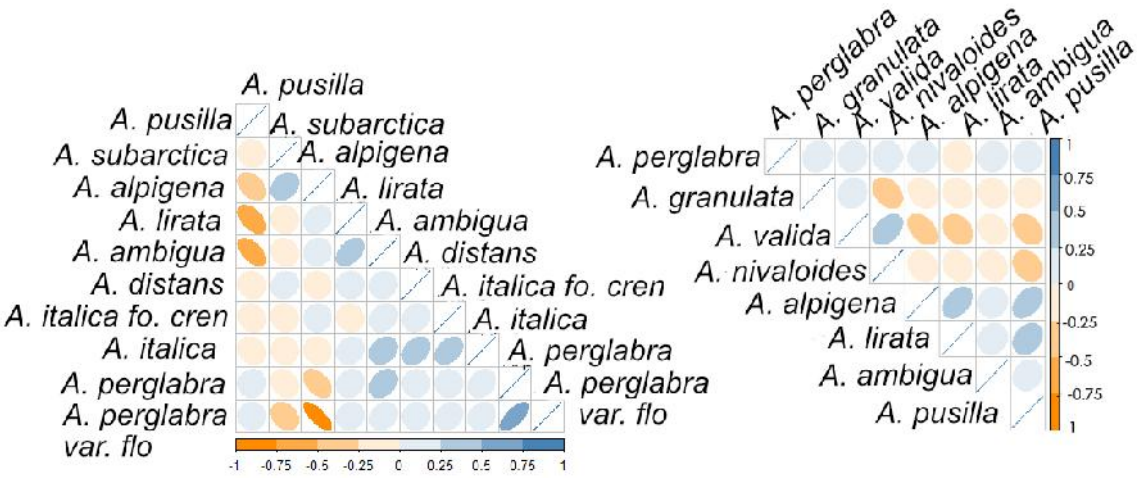

Figure 4.4 Correlogram of the occurrence of Aulacoseira species (A) from Beauty core and (B) from Kersey core.

***Aulacoseira alpigena* (Grunow) Krammer 1991**

Basionym: *Melosira distans var. alpigena* Grunow

The taxon has previously been reported as *Melosira distans* var. *alpigena* Grunow 1882, *Melosira italica* var. *alpigena* (Grunow) A.Cleve 1934, *Aulacoseira distans* var. *alpigena* (Grunow) Simonsen 1979, *Aulacoseira lirata* var. *alpigena* (Grunow) E.Y.Haworth 1990, and *Aulacoseira distans* var. *septentrionalis* (Camburn & Charles 2000) (Guiry & Guiry, 2020).

Valve diameter 6-9 µm, mantle height 3-6µm and height-diameter ratio of 0.5. Valve circular with straight chains formed by two frustules. Valvar face flat and without areolae. Margin rim punctae produced by long, thin, marginal separating spines. Curved striae formed by 6-12 punctae (**Figure 4.5** a-c).

This species was the most abundant taxon in Beauty and Kersey Lake sediment cores. In Beauty Lake, it was found all along the core, with a decreasing trend from bottom to top. Lowest relative abundances were during the 834 to1039 CE and in the top of the core from 1902 to1983 CE (**Figure 4.2**). In Kersey Lake sediments, the trend was opposite to that in Beauty Lake sediments. It increased from bottom to top, though was absent from depths 51 to 54.5 cm (**Figure 4.3**).

The decreases in relative abundance of *A. alpigena* had a strong negative correlation with *A. perglabra* var *florinae* (ρ=-0.75 to -1, $p < 0.01$) and a weak negative correlation with *A. pusilla* (ρ=-0.25 to -0.5, $p < 0.01$). For Kersey Lake it was weakly positively correlated (ρ<0.25) with *A. lirata* ($p < 0.001$) and *A. pusilla* ($p < 0.001$)(**Figure 4.4**).

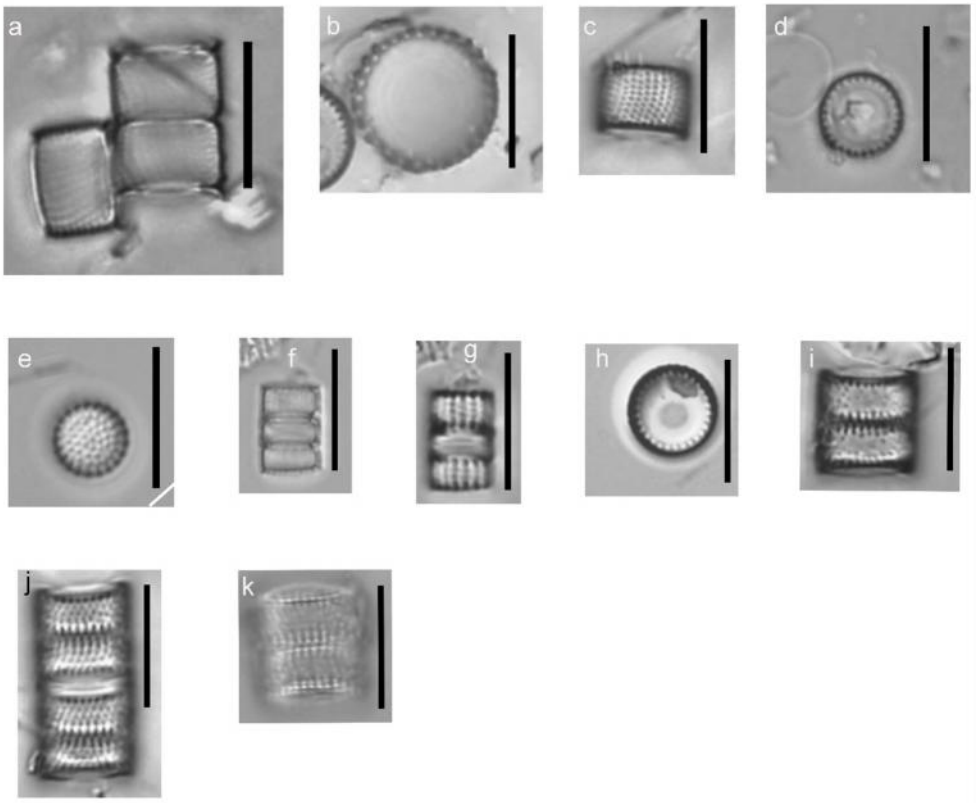

Figure 4.5 Images of *A. apigena, A nivaloides* and *A. peglabra* var. *florinae*.

a-c images of *A. alpigena*, d-g pictures of *Aulacoseira nivaloides,* h-k *Aulacoseira perglabra* var *florinae*. a. Contour of *Aulacoseira alpigena* (Grunow) Krammer 1991 sister valves. b. Valve face of *A. alpigena*. Smooth surface, lacking areolae and rim punctate. c. Girdle view of *A. alpigena* showing curved striae pattern and spines. d. Inner view of *Aulacoseira distans* var *nivalis.* e. Valve face of *A. nivaloides* (K.E Cambrum) J. English & Potapova 2009 with sparse randomly spaced areolae distribution. f and g. Frustule of *A. nivaloides* at different focal planes. Note the sulcus and the straight pattern of the puncta row in the mantle. h. Valve face of *Aulacoseira perglabra* var. *florinae,* i. Sister valves of *Aulacoseira perglabra* var. *florinae*
j. *Aulacoseira perglabra* var. *florinae* chain k. *Aulaocseira perglabra* var. *florinae* contour Scale bar in LM pictures = 10um

Aulacoseira nivaloides (K.E.Camburn) J.English & M.Potapova 2009

Basionym: *Melosira distans var. nivaloides* K.E.Camburn

It has also been known as *Aulacoseira distans var. nivaloides* (Camburn) E.Y.Haw (Jonathan English & Potapova, 2009).

Frustules were only observed in the Kersey Lake core. The diameter ranges between 4 to 6µm, mantle height 3-5µm and height-diameter ratio 0.6. Valve face showing randomly spaced areolae and short ringleiste. Mantle ornamented by straight striae formed by 4-6 areolae (**Figure 4.5** d-g).

This species was only found in the Kersey Lake core, in which it had high relative abundances. The highest relative abundance was found close to the bottom of the core and decreased towards the top of the core. The species was only absent from 43.5 to 50.5 cm. The largest relative abundance was found between 55.5 to 60 cm, where it reached ~30% (**Figure 4.3**). This taxon was negatively (<-0.25 to -0.5) associated with all the *Aulacoseira* taxa found in this core, except *A. valida* (ρ= 0.25-0.5, $p < 0.001$) and *A. perglabra* (ρ= <0.25, $p > 0.05$). Significant associations were found with *A. alpigena* ($p < 0.05$), *A. lirata* ($p < 0.05$), and *A. pusilla* ($p < 0.01$).

***Aulacoseira perglabra* var *florinae* (Camburn) E.Y.Haworth 1990**

Basionym: *Melosira perglabra* var. *floriniae* Camburn

Diameter of the frustules of the specimens found in Beauty Lake sediments is on average 8.35 µm with 4µm of mantle height, and height-diameter ratio of 0.5. Valve face is flat and hyaline, trim striate. Valve margin serrate by short spines. Short mantle, with striae composed by 3-4 areolae (**Figure 4.5** h-k). The presence of a single ring of areolae on the periphery of the valve face is usually used to identify this variety; however, this trait was not observed in the material used to identify this specimen (Camburn & Charles, 2000; Siver & Kling, 1997). The specimens found in this lake did not show valvar face with this trait.

In Beauty Lake sediments, this taxon began appearing prior to the onset of the Medieval Climate Anomaly (~835 CE), and peaked between 1902 to 1983 CE (**Figure 4.2**).

4.5 Discussion

The specimens of *A. pusilla* found in Beartooth Mountain lakes sediments suggest that North American populations are more widely distributed than previously reported, which included Eastern North American ecosystems (M. G. Potapova et al., 2008) and temperate reservoirs in South America (Costa et al., 2017). The valves in the Beartooth lakes had a larger diameter than those usually found in North America and were on the small side of the height range reported for Japanese environments (A Tuji & Houki, 2004; Akihiro Tuji, 2015). These changes in dimension are associated with size decrease and restoration during reproduction events (D. H. Jewson & Granin, 2015). Hence, every cell division generates narrower cells (D. Jewson, 1992). In nutrient-rich conditions, vegetative reproduction is stimulated, producing smaller cells. Different species of *Aulacoseira* have shown diameter changes associated with nutrient availability. Studies on *A. alpigena* and *A. islandica* diameter indicated that they respond to nutrient-rich environments. However, for *A. islandica, the* variation is not significant (Turkia & Lepistö, 1999). Even though valve changes are species-specific (D. H. Jewson & Granin, 2015), the wider frustules observed in these alpine lake sediment records could reflect the oligotrophic conditions that characterized these lakes and long life cycles.

Peaks in the relative abundances of *A. pusilla* in recent sediments of both Beauty and Kersey lakes and during times associated with warm conditions (MCA) in Beauty Lake suggests that *A. alpigena* and *A. pusilla* have ecological differences. Peaks in the relative abundance of the genus *Aulacoseira* are often associated with deep mixing. *A. alpigena* indicates strong mixing, thus cool water periods (Stone 2016). However, refined taxonomy showed a different fossil profile in Beauty Lake in the last 2000 years (**Figure 4.6**). Although *A.*

alpigena peaked in cool times, increases in the relative abundances of *A. pusilla* did not necessarily reflect temperature but snowpack variation. Indeed, the occurrence of this taxon during negative precipitation anomalies would be indicative of water column changes related to light access (Chapter 2). A similar response was suggested for *A. subarctica* in lake sediment records spanning the Holocene in Kamchatka. It was attributed to shifts in the light environment affected by winter precipitation (Solovieva et al., 2015). *A. baicalensis*, a taxon thriving under ice, showed higher densities when deep snow decreased, due to deep light penetration in the water column. Hence, the presence of *A. pusilla* during warmer times may indicate thinner ice layers from less snow accumulation. Therefore, more energy and light can reach the water column (D. H. Jewson et al., 2009).

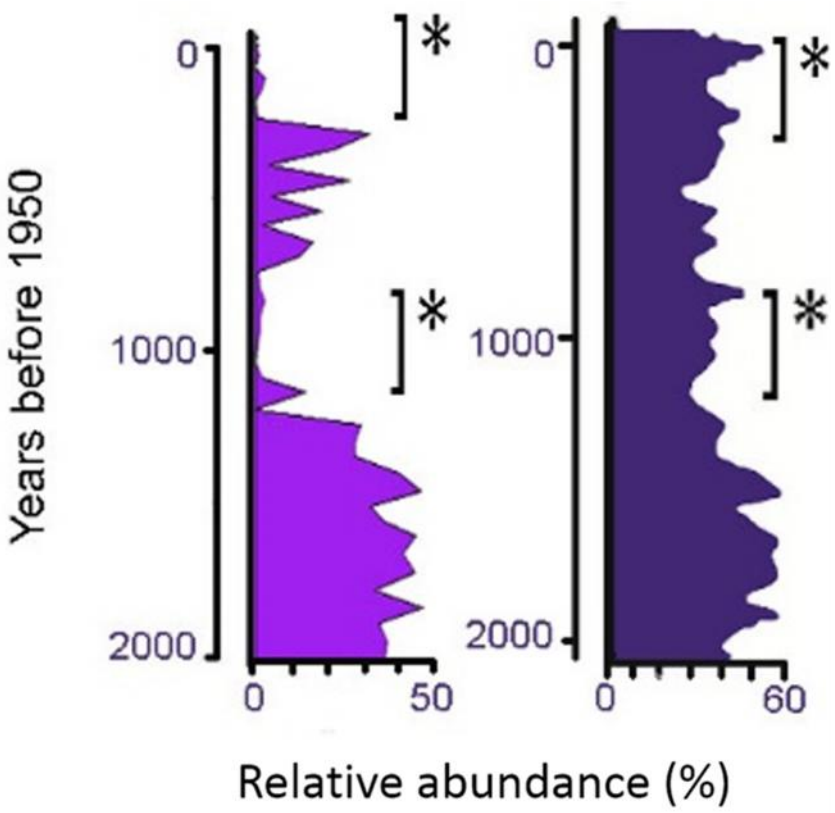

Figure 4.6 Comparison of the chronological profile of *Aulacoseira alpigena* in Beauty Lake for the last 2000 years after refining the taxonomy.

a) Observed distribution after refining the taxonomy; b) original distribution of the taxon. Asterisks point out the periods when the relative abundance of *A. alpigena* was revised due to *A. pusilla* identification. Graph adapted from *Stone et al*.2016.

A refined taxonomy of the *A. alpigena* complex in Kersey and Beauty Lake led us to think that we might see a similar trend in the other alpine or subalpine lakes of the area (Jasper, Albino Emerald, Hidden, and Rock Island lakes). However, we did not find *A. pusilla* in other fossil records. The lakes in this study are in the same ecoregion and are oligotrophic; hence they

are exposed to similar regional conditions and do not span a large trophic range. In addition, *A. pusilla* has been reported in ecosystems of differing trophic status, from oligo- to meso- and eutrophic conditions (Chen et al., 2014; Denys et al., 2003; Schroeder et al., 2016; Akihiro Tuji, 2015; Williams et al., 2016a). The similarity of basin conditions of the lakes also suggests that lake morphology is not responsible for the absence. An elevational gradient is known for influencing diatom diversity (Soininen & Teittinen, 2019). However, the lakes in this study covered a wide range of altitudes, and the lakes lacking *A. pusilla* are in elevations lower or higher than Kersey and Beauty Lake; therefore this factor is unlikely the driver of the observed distribution. Hydrological connectivity can affect the dispersal of diatoms, but Beauty and Kersey are not connected to each other. Environmental heterogeneity at the basin scale is likely affecting the observed distributions (He et al., 2020). The study of the fossil diatom assemblages in Beauty Lake suggested that *A. alpigena* and *A. pusilla* are linked to SWE variations (Chapter 2), thus watershed features such as slope, bare ground, or the susceptibility to wind may play a role in shaping how snowpack and the lake ice layer are regulated by wind action and heat exchange (Livingstone, 1997; Smits et al., 2020).

The presence of *A. alpigena* often indicates deep mixing periods and cooler climate (Stone et al. 2016). Thus, under warmer conditions it decreased in contemporary sediments (Stone et al., 2016b; Weckström et al., 2015). However, it increased in recent sediments in Kersey Lake. The association of this taxon with temperature and SWE records in Beauty Lake indicated that relative abundances of *A. alpigena* increase with moderate SWE values (Chapter 2). Thus, with less snowpack, a decrease in relative abundances was expected for this species, but the opposite trend in Kersey Lake suggests that the impact of changes in ice and snow on *A.*

alpigena depends on lake characteristics. Ice cover and snow accumulation reduce the amount of light reaching the water column and the access that stimulates or inhibits *Aulacoseira* growth (D. H. Jewson et al., 2009). Under warmer scenarios, snow thaw and early ice-out might negatively affect this taxon in the clear waters of Beauty Lake, exposing it to high light for more time. In contrast, as Kersey Lake is less transparent than Beauty Lake, extended light access may favors this species. Thus, prolonged light exposure in the highly transparent Beauty Lake might induce inhibition, whereas longer light access in Kersey Lake may contribute to the proliferation of this taxon.

The recent decline in relative abundances of *A. nivaloides* in Kersey Lake sediments indicates that modern conditions are less favorable for this taxon in this lake. A similar trend was reported in Fog Lake, an arctic lake that had a constant presence of the taxon until 1970, when rising temperatures were recorded (Weckström et al., 2015). Other studies have reported it in oligotrophic glacial and boreal lakes, characterized by low temperatures (Ilmavirta & Kotimaa, 1974b; Miettinen, 2005; Stefanova et al., 2003).

Finally, *A. perglabra* var *florinae*, which peaked during the last millennium in Beauty Lake, has been observed in oligotrophic and acid waters of a boreal forest lake (Anderson et al., 1996). In Beauty Lake sediments, the relative abundance of this taxon was higher under lower SWE values, while in Kassjön Lake in Sweden, it declined around the MCA (Anderson et al., 1996).

The correlations observed between the species in Beauty and Kersey Lake sediments are striking due to the contrasts that they showed. For example, *A. pusilla* in Beauty Lake was moderately and negatively associated with *A. alpigena* and *A. lirata*, while in Kersey Lake the links were positive. On the other hand, A. *alpigena* had moderate to weak links with most of the taxa. The inconsistency in the links between the species suggests that the diatom assemblages in both lakes are susceptible to local drivers that regulate species distributions individually as well as the interaction between species, as the interaction between the same species in these lakes do not show a clear pattern.

4.6 Conclusions

The use of refined taxonomy for the study of *Aulacoseira* revealed that the richness of this genus in the lakes analyzed is higher than expected, opening the possibilities for further climate studies using these taxa. The taxonomic revision of lake sediment records in the Beartooth Mountains showed that only two out of seven lakes have *A. pusilla* present. This suggests that the distribution of this taxon in the area may be limited. However, this limited distribution presents the opportunity to explore the reasons behind the distribution of this species in a small area. The temporal distribution patterns of *A. pusilla* revealed that the success of this species may be strongly linked to reduced SWE, however the reduced strength of this link in Kersey Lake and contrasting links of the taxon with other species suggest that the influence of local factors plays an important role in shaping the response of this taxon.

We think that the results of this study are the seeds to develop further studies of the genus *Aulacoseira* given the contrasts observed. This study raises questions about the factors that determine the success of *A. pusilla* in alpine lakes and regulate the interaction with other species. Additional research on the genus will clarify questions about the niches of these species, which will represent a significant advance for environmental reconstructions.

5 CHAPTER 5 CONCLUSIONS

The purpose of this dissertation was to decipher the factors that regulate the distribution and occurrence of *Aulacoseira* species in contemporary ecosystems and to identify if changes in the relative abundances of these species in paleolimnological records can be associated with changes in snowpack. Environmental reconstructions with *Aulacoseira* use changes in relative abundances of this group to identify changes in the stability of the water column. However, contradictions about the conditions around the occurrence of these taxa are affecting the accuracy of interpretations. As much of the water column stability depends on snowfall and snow accrual, it is essential to establish if there are connections between these diatoms and snowpack shifts. Most of the conflicts in environmental reconstructions are around low-height mantle species, which represents a significant component of the aquatic communities in alpine lakes. The experimental portion focused on the influence of light and nutrients in the development of *A. pusilla* populations, while sediment analyses explored the climatic connections.

The first part of the dissertation centered on the factors that regulate the distribution of *A.pusilla* populations in the water column. The research generated breakthroughs that provide pieces of evidence to correct some conceptions about *Aulacoseira,* in particular about the ecology of *A. pusilla.* The first aspect is about the location of the population in the water column under stable conditions, secondly the role of nutrients in the development and growth of populations, and lastly how light shapes the blooms of the taxon. The first chapter addressed this aspect by the study of temporal and spatial variation. The current assumption about mixing

is that it is important to activate resting cells and incorporate them to the phytoplankton community. With much of the data collected in stratified conditions, we expected to find *A. pusilla* migrating downward once the stratification started. We found that the stratification offers a set of intricate conditions that shows that sedimentation is not straightforward. Instead, it is an interplay with nutrient concentration, which influences the location of *A. pusilla* in the water column. A deeper study about the location of similar species in the water column may permit us to extrapolate the conditions of the migration pattern to the species in the genus *Alulacoseira*, given that sedimentation is a process that affects *Aulacoseira* taxa.

The experimental portion of the first chapter partially contributed to address doubts about the sensitivity of the taxon to nutrient availability. Contrasts between the populations studied showed that nutrient response is population specific and depends on the conditions of the lakes. This is quite a significant finding because according to our results, many of the assumptions about nutrients can only be considered in regard to specific circumstances. So the reaction of *A. pusilla* observed in one lake could not be a norm for the taxon in all ecosystems. Considering these results it is possible that *A. pusilla* may not be a proxy to identify nutrient enrichment.

The experiments with light were the last piece of evidence that changes paradigms. Light could be considered the quintessential requirement that determines the growth and persistence of the taxon before stratification. We found that light does not operate in the classical way that it is explained: “light access, thus growth”. We found that the effect is modulated by water column characteristics and the nutrient availability to which cells have

been exposed. Thus, depending on the characteristics of the water, light access does not necessarily mean higher growth.

The second part of the dissertation focused on the paleoecological connections between *A. pusilla* and climatic fluctuation. This analysis generated important conclusions about the sensitivity of *A. pusilla* to the lake changes induced by snowpack shrinking and early ice-out. The finding of links among the taxon and low SWE suggests if warm trends continue, it is possible that sedimentary records in many lakes will show an increase in the relative abundance of the species. From these results many of the queries move towards the effect on contemporary ecosystems. What could be the potential effect of the increase in the abundance of this taxon in aquatic communities? Could this induce a bottom up effect on other organisms? Despite all these questions, what cannot be forgotten is that observed pattern may occur only in lakes like Beauty Lake. In ecosystems like Kersey Lake, with warmer, less transparent waters and forested basins, the impact could be different or minimal, as the fossil records showed a more diverse assemblage in which *Aulacoseira* was not the main component.

The use of refined taxonomy also contributed to reduce the gap about *Aulacoseira* ecology. Studies that group many of the low-mantle species into the *A. distans* complex assume ecological similarities among the morphospecies. The contrasts observed in the distribution of *A. alpigena* and *A. pusilla* in the fossil records in Beauty Lake showed that morphological similarities do not mean ecological resemblance. Detailed observations of the assemblages could reveal additional difference about the ecological conditions of these species. Hence, it would reduce the discrepancies in the interpretation and reconstructions of the past.

Finally, the differences for *A. alpigena* in Kersey and Beauty Lake sediments showed a remarkable aspect that we did not expect to observe. Despite how rising temperatures affect snowpack and lake insulation, the impact for *A. alpigena* differed among lakes. This means that this taxon may only be a proxy for deep mixing in highly transparent lakes; additional autecological work with this taxon will help to clarify this aspect.

In summary, this research concentrated on the study of *A. pusilla* ecology, and possible links with snowpack. The study generated important insights for the ecology *A. pusilla.* The taxon varies in spatial and temporal distribution as the lakes stratify. We expected a homogeneous distribution as the population reached deeper depths. Instead, we found that this process is regulated by the availability of nutrients and light, which can be a strategy of *A. pusilla* to extend its presence in the water column and exploit the resources still available at shallow depths before the lake reaches the stability. However, much of this response is modulated by the conditions of light. The growth of this taxon is stimulated by nutrients, if it has been previously exposed to nutrient depletion, but if nutrients are sufficient, there will not be an effect. As we saw with nutrients, light is a critical factor for populations, but the stimulus occurs if previously they faced light reductions like in Kersey Lake. Incubations in deeper layers suggested that temperature could be a third factor controlling growth, which suggests that additional studies are required to identify the sub-optimal conditions. The sediment analysis showed that *A. pusilla* is favored by light access, it blooms in times identified as warm; therefore, we think that expanding the analysis to other lakes where this taxon is present may strengthen the link we found with low snowpack conditions. While the distribution of the taxon was limited in similar lakes in the area, further studies of the characteristics and differences

where the species has been reported will provide information about the niche of *A. pusilla*. As the data for this research came from the GYA, our results represent a perk for the conservation of the area. High-elevation ecosystems in the Central US Rockies are considered less vulnerable to warmer conditions. We found that the association with snowpack decrease induced changes in the diatom assemblages of the lakes analyzed.

The results of the study not only represent an advance in the understanding of *A. pusilla,* but many of the observations like factors that determine the distribution in the water column may apply to other species in the *A. distans* complex, and even to the genus given that lake turnover and sedimentation are processes that are part of the life cycle of the genus, thus some of the conclusions in this work can be extrapolated. We believe that many aspects of the patterns of fossil distribution should move the discussion to understanding whether the changes at the diatom level have induced changes at higher trophic levels of aquatic communities in the GYA, and how this can affect the ecosystems.

6 REFERENCES

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7 APPENDICES

7.1 APPENDIX A. RELATIVE THERMAL RESISTANCE (RTR) FOR KERSEY BEAUTY AND BEARTOOTH LAKE OVER THE STUDY PERIOD

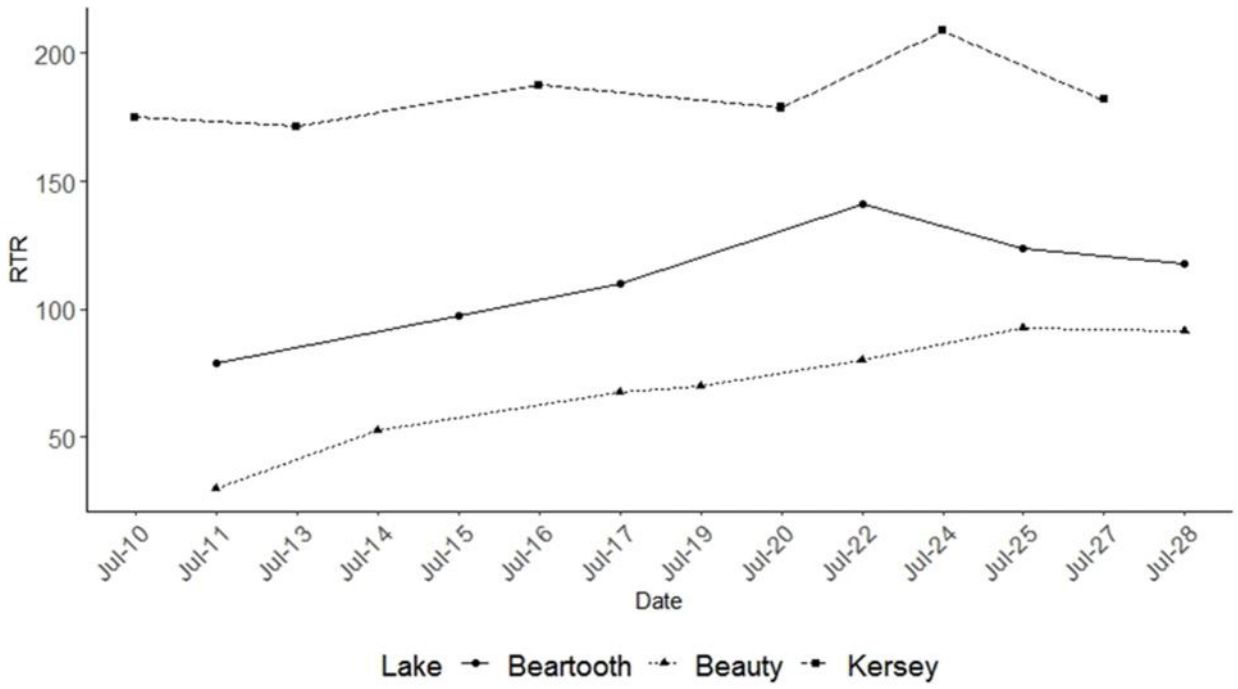

Figure 7.1 S1.Values of Relative Thermal Resistance for the lakes over the study period.

7.2 APPENDIX B. NUTRIENT CONCENTRATIONS AND RATIOS IN KERSEY, BEAUTY, AND BEARTOOTH LAKES

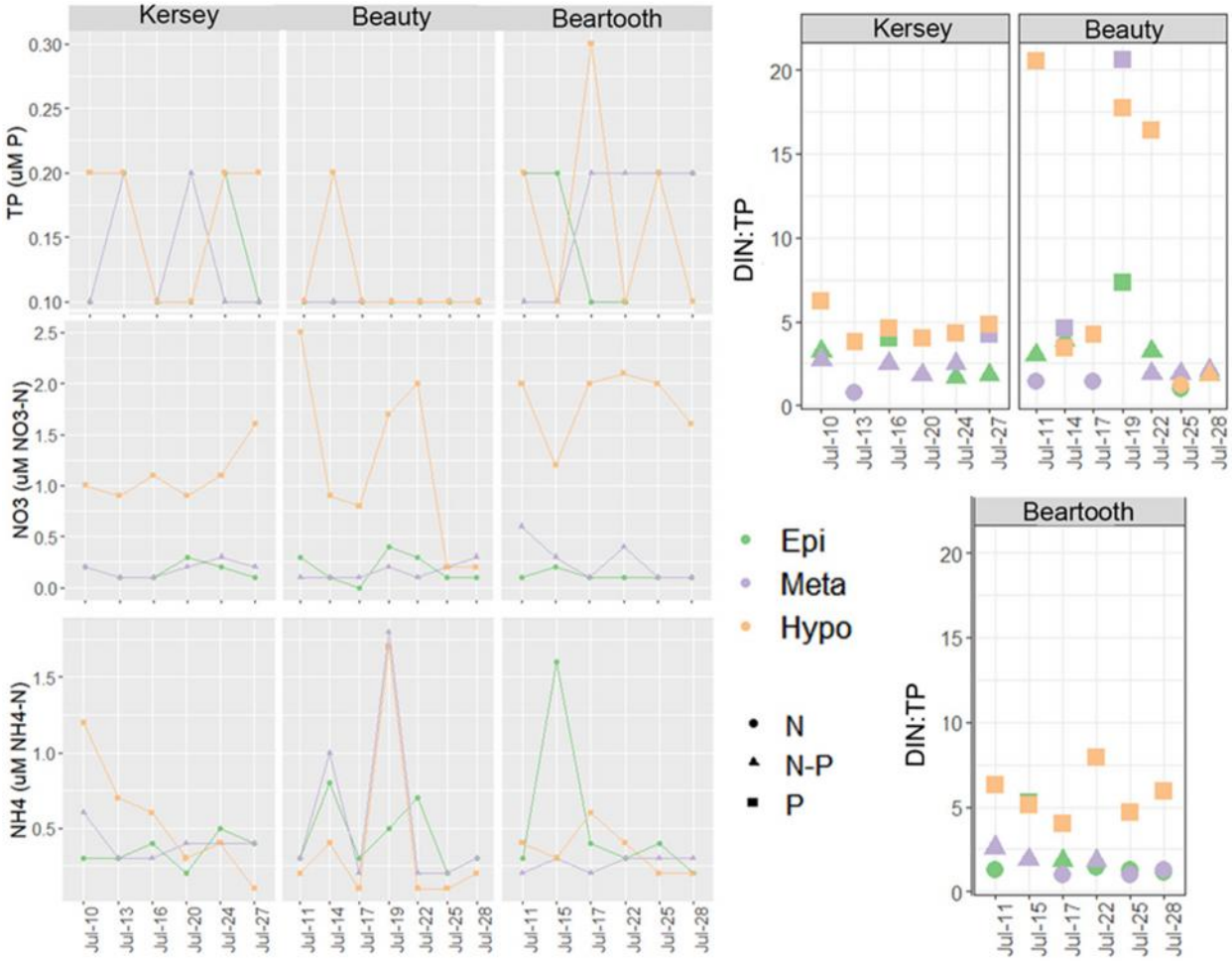

Figure 7.2 Nutrient concentrations and ratios in Kersey, Beauty, and Beartooth lakes.

DIN:TP ratio calculated using concentrations $\mu g l^{-1}$. Colored lines indicate the zone of study; closed symbols indicate the limiting nutrient.

7.3 APPENDIX C. PHYTOPLANKTON COMPOSITION FOUND IN KERSEY, BEAUTY, AND BEARTOOTH LAKES

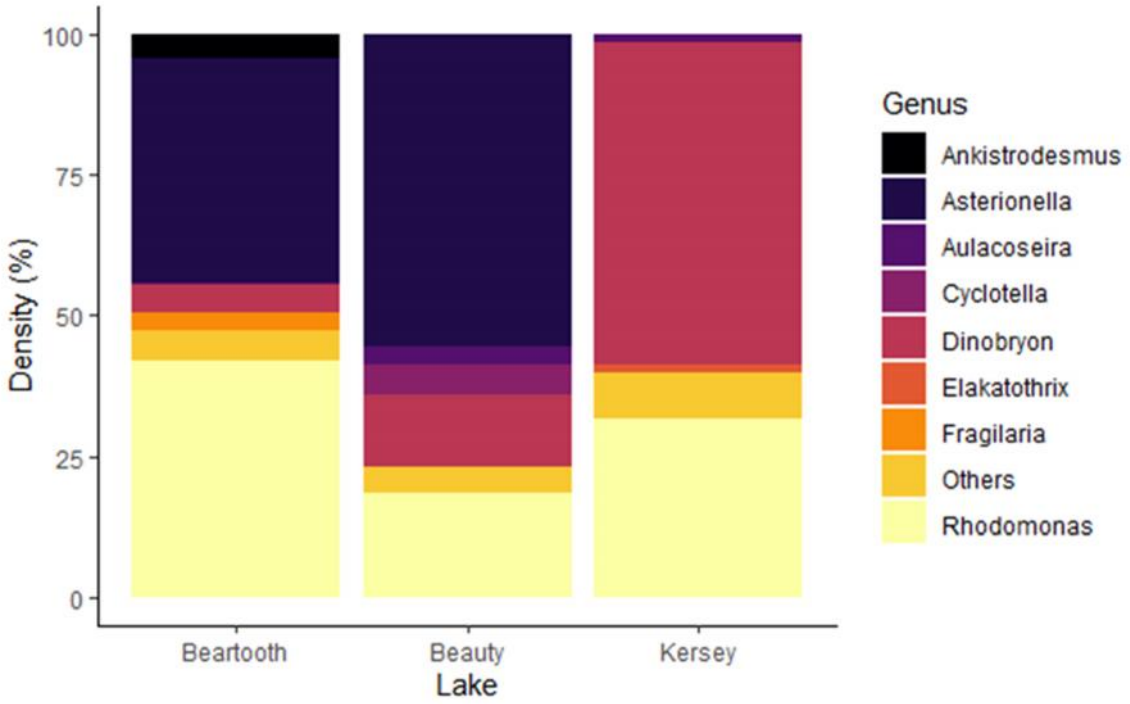

Figure 7.3 Phytoplankton composition found in Kersey, Beauty, and Beartooth lakes over the study period. Density reported as percent of the total density.

7.4 APPENDIX D. SUMMARY OF FITTED MODELS FOR *A. PUSILLA* DENSITY IN KERSEY, BEAUTY, AND BEARTOOTH LAKES

Table 7.1 SI. Summary of fitted models for *A. pusilla* density in Kersey, Beauty, and Beartooth lakes.

Conductivity (SPC), and average light in zones (PAR), degrees of freedom (df).

	Kersey Lake	Beauty Lake	Beartooth Lake
Model	Density= 5.06 + 9.7E (PAR)	Density= 84.5- 0.99(PAR)	Density= 4.6E0.6 – 6.7(SPC) – 9.95(PAR)
Intercept p-value	<0.001***	<0.001***	<0.05**
PAR p-value	<0.05**	<0.001***	0.01*
SPC p-value	NA	NA	8.9E-3**
Null deviance	90.1 on 17 df	709.2 on 20 df	100 on 17 df
PAR deviance	53.6 on 16 df	189.7 on 19 df	42.5 on 16 df
SPC deviance	NA	NA	98 on 16 df
Dispersion for Quassi-Poisson	3.1	10.3	3
R^2	0.38	0.65	0.41
Goodness of fit (p-value)	6E-0.6	1.9E-4	0.42

BIOGRAPHY OF THE AUTHOR

Edna Luz Pedraza Garzón was born in Bogotá, the capital city of Colombia. She graduated from San José School and got her degree in 1999. In 2000 she was accepted to the Bachelor program in Biology at the Pontifical Xaverian University, Bogotá sectional, and graduated from the program in 2005. Three years later, Edna moved to Bucaramanga city, where she got the degree of Specialist in Natural Resources preservation and conservation in 2014 at the Pontifical Bolivarian University.

As part of her professional experience, Edna was part of the Young Research Program in the Pontifical Xaverian University, analyzing the diatom community of the Coffee Ecoregion in Colombia. She also worked in the Universidad Nacional de Colombia, studying the effect of global warming on rivers. After a year of working in consultancy, the author moved to Bucaramanga to work in the Colombia Petroleum Institute, where Edna worked for five years on a project to study the diatom flora of Colombia. Product of her participation in these projects, she is co-author of different publications about the diatom flora in Colombia.

In 2015 Edna won the Colciencias-Fulbright Scholarship that allowed her began her doctoral work at the University of Maine in June 2016. She is a candidate for the Doctor of Philosophy degree in Ecology and Environmental Science from the University of Maine in May 2021.